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THE TREATMENT OF HYPOGENITALISM IN THE MALE WITH THE GONADOTROPIC PRINCIPLE OF PREGNANT MARES' SERUM

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IT IS NOW generally agreed that the anterior pituitary-like hormone from pregnancy urine and placenta are of definite value in the treatment of some types of hypogenitalism and cryptorchidism. Also, some enthusiasm has been shown relative to the correction of these conditions by the administration of beef and sheep anterior pituitary extracts. However, there are definite limitations to the value of these preparations. The urinary and placental extracts are not true pituitary gonadotropic hormones (they fail to prevent atrophy of the gonads in the hypophysectomized animal), and therefore cannot actually be considered substitution therapy. In the female, the principal effect of these extracts is that of luteinization of the ovaries, and in the male, stimulation of growth of the interstitial tissue of the testes. Success in the treatment of some cases of cryptorchidism may possibly be explained by the increased weight of the testes, or by growth and elongation of the supporting structures produced by the hormone. Theoretically, the pituitary extracts should be ideal preparations for the correction or improvement of sexual disturbances due to insufficiency of the anterior lobe of the pituitary. However, as previously mentioned, in the hands of some investigators the pituitary extracts have met with limited success. The extraction and concentration of the anterior lobe gonadotropic hormone is difficult and the potency and stability of many commercial extracts is unreliable. Furthermore, the very source of these preparations make their supply more or less limited and the price exceedingly high. For these reasons, other sources of potent gonadotropic factors, pituitary-like in physiological properties are desirable.

In 1930, Cole and Hart (1) found that during a limited period of pregnancy, there is present in the blood serum of mares, a high concentration of gonad stimulating substance. When the pregnancy serum is injected into immature animals the gonads

change from the immature state to that found in mature animals. Immature male rats injected with mares' serum showed at autopsy, 4 to 5 days later, an increase in the size of the testes and an even greater response in the seminal vesicles and prostate. In the female rat, the ovaries increased in size with many mature follicles and corpora lutea; also stimulation of the uterus and production of a positive estrous reaction resulted. Experiments on larger domestic animals resulted in comparable results. Hart and Cole (2) found that the hormone increased the activity of the Leydig cells of the testes and produced enlargement of the seminal vesicles and prostate. Meyer and Gustus (3) produced follicle stimulation and development of the secondary sex characteristics in immature rhesus monkeys.

The gonadotropic hormone of mares' serum does not act directly on the tubular tract (uterus and vagina) but exerts its stimulating effect on the ovaries producing follicle and corpus luteum development, which is followed secondarily by estrous changes in the tubular tract, characteristic of the ovarian hormones which are produced in the ovary under stimulation by the gonadotropic hormone.

Davis and Koff (4) have recently shown at operation the effectiveness of mares' serum in producing follicle maturation, and have successfully treated certain cases of female sterility with this hormone.

Biological studies on the gonadotropic hormone of mares' serum have established certain differences between it and the gonadotropic hormone from pregnancy urine, placenta and the anterior lobe of the pituitary. Goss and Cole (5) have shown that unlike the hormone found in the blood and urine of pregnant women, mares' serum hormone even in its highest concentration in the blood stream at about the 65th day of pregnancy is not excreted in the urine. They also have demonstrated differences between mares' serum and pregnancy urine on immature male birds and hypophysectomized male animals. They concluded that the biological effects produced by the mares' serum hormone when injected into animals are more of the nature of a physiological response than in the case of pregnancy urine. In this respect, the gonad stimulating action of mares' serum more closely resembles the gonadotropic effects produced by the animal's own hypophysis.

In view of the remarkable gonad stimulating properties of mares' serum, (resembling in biological effects those of the anterior lobe extract) in experimental and domestic animals, and because of the convenient source of the hormone, it seemed justifiable to demonstrate the clinical effects of the hormone on patients with hypogenitalism and cryptorchidism.

MATERIAL FOR STUDY

Fourteen boys between the ages of 6½ and 14¾ years, formed the group for this study. Each patient was given a thorough physical examination including measurements. Roentgenograms of the sella turcica and of the extremities for bone age were made; results of B.M.R., dextrose tolerance test, serum cholesterol, Wassermann and Kahn tests, complete blood count and urinalysis were recorded before treatment was instituted. Photographs of each patient were taken before and after treatment.

The material used was pregnant mares' serum hormone prepared by the Upjohn Company,[1] a protein purified extract containing a maximum of 0.04 mg. of protein nitrogen per 10 Upjohn rat unit tablet. In view of the fact

[1] Gonadogen. The Upjohn rat unit is the total dose of hormone which, divided into 3 daily subcutaneous doses in 21 to 23-day-old female rats weighing 30 to 40 gm. will produce at autopsy on the 5th day a pair of ovaries weighing 65 mg. (5 times greater than uninjected control.)

that the mares' serum hormone, urinary prolan and the anterior lobe extracts differ from the standpoint of their origin and their physiological activities in animals, they cannot be compared quantitatively. Before administering the hormone, each patient was tested intradermally with 1/10 cc. of the diluted hormone (1 ten-unit tablet dissolved in 1 cc. of normal saline). The test was observed for one-half hour and if no marked local reaction occurred the hormone was then administered intramuscularly in the full dose. None of the 14 patients had marked local reactions from the intradermal test, the most pronounced being that of a one centimeter wheal with one millimeter elevation in only one patient.

From 10 to 20 U.U. were administered intramuscularly 3 times weekly. (One patient, *Case* 3, received 40 U.U. 3 times weekly for 3 months). Rest periods from 2 to 3 weeks or longer were given to several of the patients because we did not know at the time whether an antibody or antihormone response would be produced in the patients under continuous treatment. The course of treatment varied from a minimum of 2 weeks to a maximum of 18 months. Some of the patients are still under treatment and this is indicated in the accompanying table (pp. 664–665).

RESULTS OF TREATMENT

The results of treatment were judged by the growth of the genitalia, appearance of secondary sex characteristics and descent of undescended testes.

Although 14 patients began treatment with mares' serum, only 12 are included in the analysis. Two patients had to be eliminated because of insufficient treatment (due to severe local reactions). The results were graded as follows.

(a) *Marked improvement:* Marked growth of genitalia with or without appearance of secondary sex characteristics; complete descent of undescended testes. There were 4 patients (33⅓%) who responded in this manner.
Case 3, 14 years old, received a total of 2550 U in 18 months
Case 4, 11 years old, received a total of 970 U in 9½ months
Case 5, 12 years old, received a total of 920 U in 11 months
Case 6, 14¾ years old, received a total of 990 U in 12 months

(b) *Moderate improvement:* Moderate growth of genitalia, partial or complete descent of undescended testes. There were 4 patients (33⅓%) who responded in this manner.
Case 9, 11 years old, received a total of 300 U in 11 weeks
Case 12, 12 years old, received a total of 240 U in 2½ months
Case 13, 13½ years old, received a total of 500 U in 5 months
Case 14, 13½ years old, received a total of 360 U in 3 months

(c) *Little improvement:* Slight growth of genitalia. One patient responded in this manner.
Case 2, 11½ years old, received a total of 480 U in 2 months.

(d) *No improvement:* Three patients (25%) showed no improvement.
Case 7, 9½ years old, received a total of 240 U in 2 months
Case 10, 6½ years old, received a total of 240 U in 2 months
Case 11, 11 years old, received a total of 520 U in 2½ months

Of the 12 patients treated, 8 or 66⅔% showed evidence of definite improvement following treatment with mares' serum. Some are still under treatment.

Effect of Mares' Serum upon Cryptorchidism

Of the 14 patients treated, there were 4 who showed pseudo-cryptorchidism in which the testis was freely movable and could be manually placed into the scrotum, but retracted immediately (*Cases* 1, 2, 5, 9). *Case* 1 re-

TABLE 1. TREATMENT OF HYPOGENITALISM IN THE MALE WITH THE GONADOTROPIC PRINCIPLE OF PREGNANT MARES' SERUM

Case No.	Age	Ht.	Wt.	Diagnosis	Clinical Findings	TREATMENT		Results	Untoward Reactions
						Dose	Duration		
	yr.	in.	lb.						
1	13	56	96	Hypogenitalism and obesity. Pseudocryptorchidism.	Shortness of stature. Pectoral and girdle obesity; hypoplastic genitalia. Testes riding in mid-inguinal canal; may be forced into scrotum; retract immediately.	10 U.U. 3×wkly. Total 50 U.U.	1⅔ wk.	Insufficient treatment	Severe local reactions requiring discontinuation of treatment.
2	11½	58¾	95	Hypogenitalism and moderate obesity; Pseudocryptorchidism. (9½ yr.-rt. orchidopexy; 10½ yr. 4 mo. course of antuitrin-S for bilateral cryptorchidism.)	Pectoral and girdle obesity; hypoplastic genitalia. Scrotum, testes, penis infantile. Rt. testis atrophic in upper scrotum. Left testis in upper scrotum hypoplastic. Both can be forced into scrotum; retract immediately.	20 U.U. 3×wkly. Total 480 U.U.	2 mo.	No increase in size of rt. testis, moderate increase of left testis. Scrotum slightly larger. No increase in size of penis. No change in obesity.	Slight local reactions on 4 occasions.
3	14	65½	164	Froehlich syndrome. True cryptorchidism.	Marked pectoral and girdle obesity; knock knees and flat feet. Bilateral cryptorchidism. Testes located at internal ring, pea sized—descend to upper scrotum with force; retract immediately.	20 U.U. 3×wkly. 960 U.U. 40 U.U. 3×wkly. 1440 U.U. 10 U.U. 3×wkly. 150 U.U. Total 2550 U.U.	6 mo. 2 mo. rest period 3 mo. 4 mo. rest period 5 wk. 18 mo.	No change in fat distribution. Growth of 2½ in. and gain of 14 lb. in 18 mo. Complete descent of both testes with marked growth of testes and scrotum. Moderate growth of penis and pubic hair.	No unfavorable reactions.
4	11	58½	105	Hypogenitalism and moderate obesity. True cryptorchidism. (4300 R.U. of A.P.L. 4 mo. previously for bilateral cryptorchidism.)	Moderate girdle obesity. Bilateral cryptorchidism. both testes hypoplastic located below internal ring. Descend to upper scrotum with force; retract immediately. Penis small.	10 U.U. 3×wkly. 110 U.U. 20 U.U. 3×wkly. 620 U.U. 10 U.U. 3×wkly. 240 U.U. Total 970 U.U.	1 mo. 2½ mo. 3 mo. rest period 3 mo. 9½ mo.	Complete descent of both testes. Growth of penis and testes. No change in fat distribution. Growth of 2 in. and gain of 14½ lb. in 10 mo. (Still under treatment.)	No unfavorable reactions.
5	12	59½	122½	Hypogenitalism and obesity. Pseudocryptorchidism. (4450 R.U. of A.P.L. at 11 yr. for bilateral cryptorchidism.)	Pectoral and girdle obesity. Bilateral cryptorchidism. Testes below internal ring; pea sized, soft. Can be forced into scrotum; retract immediately. Penis and scrotum small.	10 U.U. 3×wkly. 200 U.U. 20 U.U. 3×wkly. 240 U.U. 20 U.U. 3×wkly. 480 U.U. Total 920 U.U.	7 wk. 3 mo. rest period 4 wk. 4 mo. rest period 2 mo. 11 mo.	No change in fat distribution. Growth of 2 in. and gain of 11½ lb. in 11 mo. Complete descent of both testes. Growth of both testes and penis. Sparse pubic hair.	No unfavorable reactions.
6	14¾	57½	90½	Non-adipose hypopituitarism with retarded growth and sexual development.	Undersized; appearance of a 10-yr-old. Infantile genitalia.	10 U.U. 3×wkly. 510 U.U. 20 U.U. 3×wkly. 240 U.U. 20 U.U. 3×wkly. 240 U.U. Total 990 U.U.	5 mo. 1 mo. rest period 1 mo. 4 mo. rest period 1 mo. 12 mo.	Growth of 2¾ in. and gain of 17 lb. Marked growth of external genitalia. 4 mo. after termination of treatment genitalia almost adult size. Secondary sex characteristics present.	No unfavorable reactions.

7	9½	53¾	83	Hypogenitalism with obesity. True cryptorchidism. (4500 R.U. A.P.L. at 8 yr. for bilateral cryptorchidism.)	Pectoral and girdle obesity. Bilateral cryptorchidism. Testes riding high in canal; hypoplastic and can be pulled into upper scrotum; retract immediately. Penis undersized.	10 U.U. 3×wkly. Total 240 U.U.	2 mo.	Testes descended to mid-scrotum but retract into inguinal canal. No growth of genitalia. (Still under treatment.)	No unfavorable reactions.
8	10½	53⅝	91	Thyropituitarism. Hypogentalism.	Hypothyroidism. Retarded growth. Serum cholesterol 380, B.M.R.—30. Genitalia infantile.	20 U.U. 3×wkly. Total 120 U.U. (Thyroid extract)	2 wk.	Insufficient treatment.	Treatment stopped because of severe reactions.
9	11	57	81	Hypogenitalism. Pseudo-cryptorchidism. (3200 R.U. A.P.L. at 10 yr. for bilateral cryptorchidism.)	No evidence of endocrine disturbance. Bilateral cryptorchidism. Both testes freely movable in mid-canal; hypoplastic; can be pulled all the way into scrotum. Retract immediately. Penis underdeveloped.	10 U.U. 3×wkly. Total 300 U.U.	11 wk.	Spontaneous descent and growth of both testes. Do not retract into canals. Penis slightly larger. Growth of 1 in. and gain of 9 lb. in 2⅗ mo. (Still under treatment.)	No unfavorable reactions.
10	6½	48½	52	Unilateral cryptorchidism. (30 injections of A.P.L. at 5½ yr.)	No evidence of endocrine disturbance. Rt. testis fixed below internal ring; hypoplastic. Mechanical obstruction. Left testis in scrotum; normal size. Penis normal size.	10 U.U. 3×wkly. Total 240 U.U.	2 mo.	No descent of testis. Occasional pain and swelling in inguinal regions following injections. Left testis and scrotum increased in size. No increase in size of penis. (Orchidopexy contemplated.)	Moderate local reactions on several occasions.
11	11	61	135	Unilateral cryptorchidism. Obesity.	No evidence of endocrine disturbance. Obesity with normal distribution. Rt. testis in scrotum, normal size. Left testis at internal ring; hypoplastic, fixed. Mechanical obstruction. Penis normal size.	20 U.U. 3×wkly. 280 U.U. 10 U.U. 3×wkly. 240 U.U. Total 520 U.U.	2⅗ mo.	Penis larger. Right testis larger. Swelling in upper left inguinal ring. Left testis larger and tender but cannot be forced into scrotum. Sparse pubic hair. Growth of ½ in., gain of 3 lb.	No unfavorable reactions.
12	12	58	98	Hypogenitalism. Obesity.	Well developed, moderate pectoral and girdle obesity. Genitalia infantile. Both testes pea sized, soft, in scrotum.	10 U.U. 3×wkly. Total 240 U.U.	2⅗ mo.	Testes larger. Scrotum larger. No increase in size of penis. No sex consciousness. (Still under treatment.)	No unfavorable reactions.
13	13½	60	104	Unilateral cryptorchidism.	No evidence of endocrine disturbance. Tall, well nourished. Right testis in mid-canal, can be pulled into upper scrotum; retract immediately. Right testis and scrotum smaller than left. Left testis normal. Penis slightly smaller than normal.	10 U.U. 3×wkly. Total 500 U.U.	5 mo.	Right testis in midscrotum, larger. (Partial descent.) Left testis larger. Increase in size of scrotum. Increase in size of penis. (Still under treatment.)	No unfavorable reactions.
14	13½	59	112	Hypogenitalism and obesity. True cryptorchidism. (Extensive course of A.P.L. at 11⅓ yr. for bilateral cryptorchidism.)	Pectoral and girdle obesity. Bilateral cryptorchidism. Right testis below internal ring; can be pulled into upper scrotum, retract immediately. Left fixed at internal ring. Penis and scrotum infantile.	10 U.U. 3×wkly. Total 360 U.U.	3 mo.	Both testes larger. Right completely descended. Left testis in upper scrotum, cannot be pulled down completely. Penis slightly larger. No secondary sex characteristics.	No unfavorable reactions. Growth of 1¼ in. and of 3 lb. (Still under treatment.)

ceived insufficient treatment and is eliminated from the study. *Case 2* did not respond well to treatment. In *Cases 5* and *9* the testes descended spontaneously and remained permanently in the scrotum.

There were 7 cases of true cryptorchidism in which the testes could not be placed into the scrotum manually (*Cases 3, 4, 7, 10, 11, 13* and *14*). In this group complete descent of the testes occurred in 2 patients, both bilateral cryptorchids (*Cases 3* and *4*). Partial descent of both testes occurred in one bilateral case (*Case 7*) and complete descent of one testis and partial descent of the other in another bilateral cryptorchid (*Case 14*). Partial descent of the testis to midscrotum occurred in one unilateral cryptorchid (*Case 13*). No descent of the testis was observed in 2 unilateral cryptorchids (*Cases 10* and *11*) following treatment. Complete descent of one or more testes occurred in 3 of these 7 patients with 2 complete failures. However, the group is entirely too small to form a definite opinion. All but one of the patients with bilateral cryptorchidism showed, in addition, definite endocrine disturbance. Response to treatment was most satisfactory in this group. None of the 3 patients with unilateral cryptorchidism showed evidence of endocrine disturbance and in only one of these patients (*Case 13*) was there definite response to treatment as evidenced by partial descent of the testis. These results bear out our previous contention that cryptorchidism is most satisfactorily treated with hormones in those patients that have a co-existing endocrine disturbance (6).

Effect on Growth of Genitalia and Secondary Sex Characteristics

Marked improvement occurred in 4 patients (33⅓%) and moderate improvement in 4 patients (33⅓%), a total of 8 patients, 66⅔%, who showed definite improvement.

From results obtained in this small series of cases it is felt that mares' serum has a definite stimulating effect upon the male gonads as evidenced by increase in size of testes, increased scrotal turgescence, increase in size of the penis or the appearance of secondary sex characteristics. The first noticeable change usually is the increase in scrotal turgescence and enlargement of the testes. Enlargement of the penis does not often occur until after growth of the testes and in all probability is secondary to the production of male hormone by the stimulated testes. However, enlargement of the penis does occur more rapidly in the older boys treated at the age when puberty usually begins. The development of secondary sex characteristics may also be hastened in the older boys.

Effect upon Obesity

There was no change in the distribution of fat and there was no weight loss in any of the patients treated. In spite of the fact that definite stimulation of sexual development occurred in some of the patients the characteristic endocrine type of obesity frequently referred to as 'pituitary obesity' did not change and in fact the obesity of some patients increased out of proportion to the rate of growth during the period of treatment. This is in accord with our previous results in the treatment of endocrine disturbances with prolan (6).

Fig. 1. *Case* 3. Age 14 yr. 2550 U.U. Gonadogen in 18 mo. (a) Before treatment. (b) Six mo. after onset of treatment. (c) Eighteen mo. after onset of treatment. Note marked growth of scrotum, testes, moderate growth of penis and sparse pubic hair.

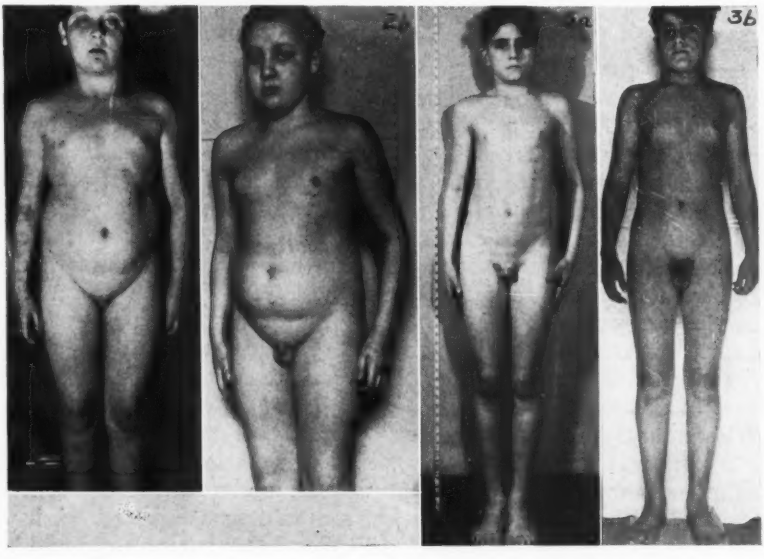

Fig. 2. *Case* 5. Age 12 yr. 920 U.U. Gonadogen in 11 mo. (a) Before treatment. (b) Eleven mo. after onset of treatment. Note complete descent of testes, marked growth of scrotum, moderate growth of penis.

Fig. 3. *Case* 6. Age $14\frac{3}{4}$ yr. 990 U.U. Gonadogen in 12 mo. (a) Before treatment. (b) Twelve mo. after onset of treatment. Note the marked growth of genitalia and pubic hair.

Untoward Effects

Of the 14 patients treated, local reactions occurred in 4 patients ($28\frac{1}{2}\%$) in spite of the negative or very mild reaction to the preliminary intradermal test. Of the 4 patients, treatment had to be discontinued in two cases (*Cases* 1 and 8) because of the severity of the local reactions. Systemic re-

actions did not occur in any of this group. However, recently a girl of 12 years developed headache, vomiting spells and a temperature of 102°, following a second injection (20 units) of this hormone.

DISCUSSION

From the results obtained in this small series of cases it is our impression that mares' serum is of definite value in the treatment of some cases of hypogenitalism and cryptorchidism. Just as any hormone has its limitations, spectacular results cannot be expected in every case. The single dose of hormone administered was a matter of personal selection, and it is now our feeling as more cases have been treated, that the individual dose should probably be increased. Greater improvement may possibly be realized in more of the patients with either larger individual doses or continued treatment. The hormone as it is now available in individual ampules of 10 U, makes it almost impossible to give larger individual doses and it is suggested for future use that the hormone be available not only in 10, but also in 20, 50 and 100-unit doses. Thus, better results or failure may be demonstrated in a shorter period of time.

From a clinical standpoint, our results were similar to those obtained with prolan and anterior lobe extracts, although with the doses of mares' serum used, the duration of treatment was more prolonged. Whether the physiological effects produced by mares' serum hormone are superior to prolan and anterior lobe extract, we cannot say because we have no comparable studies in the human male available. Davis and Koff (4) have demonstrated in the female at operation marked ovarian follicle stimulation following intravenous injections of mares' serum (which is not produced by prolan). Unless the testes are examined microscopically after injection of any of the hormones, a true comparison cannot be made. Unfortunately, this experiment cannot be carried out in the male. The only disadvantage of mares' serum that is apparent is the local reaction that not infrequently occurs. On the basis of total nitrogen determinations, the protein factor has been removed (8), but unquestionably there is some chemical responsible for these reactions.

Recently Hartman (7) was able to produce over-stimulation and injury to the ovary in the rhesus monkey. Although the injury was temporary and recovery was prompt and complete, these results must receive serious consideration in treating the human being, even though at present we have no way of knowing whether the human ovary or testes may be injured by excessive doses of the hormone. Hartman also brings out the fact that it is difficult to estimate a correct dosage as the response of any hormone varies in different individuals and in different age groups.

Treatment is being continued in some of our patients as indicated, and new patients are enlarging our group for further study. We are conducting tests on the serum of treated patients for the presence of antibodies and antihormones (9).

SUMMARY

Fourteen boys between the age of 6½ and 14¾ years, who presented hypogenitalism, cryptorchidism, or both, were treated by the intramuscular administration of pregnant mares' serum.

Definite improvement as evidenced by growth of the genitalia, the appearance of secondary sex characteristics or descent, partial or complete, of undescended testes occurred in 8 or 66⅔%.

Cryptorchidism was present in 11 of the 14 patients; pseudocryptorchidism in 4, and true cryptorchidism in 7 patients. In the latter group complete descent of the testes occurred in one or both testes of 3 and partial descent in 2 patients. Two cryptorchids did not respond to treatment.

There was no change in the distribution of fat or weight loss in any of the patients.

It is believed that mares' serum is of definite value in the treatment of certain cases of hypogenitalism and cryptorchidism.

As yet the optimal effective dose has not been established, but we believe that 50 to 100 U intramuscularly 3 times weekly probably will be more effective than the 10 to 20 U intramuscularly 3 times weekly administered to our patients. With the higher dosage results would probably be obtained in a shorter period of time.

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STUDIES ON REPRODUCTION IN THE MONKEY AND THEIR BEARING ON GYNECOLOGY AND ANTHROPOLOGY[1]

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THE OBJECT of this communication is to survey some studies that have been made on the physiology of reproduction in the monkey and to make an inventory of some of the facts of major significance which seem to have been useful to the gynecologist, student of human reproduction, on the one hand, and of interest to the anthropologist, student of human phylogeny, on the other.

I. GENERALIZATIONS OF SPECIAL INTEREST TO GYNECOLOGY

The Physiology of Menstruation

It has been known since Saint-Hilaire and Cuvier (1) that monkeys menstruate at intervals corresponding to the human cycle. It required a century to prove that of all mammals it is only the primates that menstruate, and nearly as long to demonstrate that the estrous (intermenstrual) bleeding of cows and bitches is not the homologue of the menstrual flow (2).

It follows, therefore, that monkeys and apes have become indispensable for the study of menstrual physiology and anatomy. Corner (3) first demonstrated the feasibility of studying the monkey in captivity. His pioneer work and Allen's (4) demonstration of the rôle of estrin in menstruation emphasized the high degree of similarity between structure and function of the human genital tract and that of primate animals. Those workers who have made first-hand observations on human material as well as that of monkeys feel, in general, that information gained from a study of sexual physiology in the higher monkeys is directly transferable to man. In the cyclic histological changes in the genital tract and in its response to hormonal stimulation no species differences of importance have been brought to light.

It is impossible in this paper to review the studies of the last two decades on the physiology of menstruation or to do justice to the pioneer workers in the field (3–6). The subject has, moreover, been recently reviewed from different standpoints (7–10).

While the 'cause' of menstruation has not yet been discovered, much has already been learned concerning the hormonal control of uterine bleeding. Indeed, it is upon the results of experiments on the monkey that gynecology

[1] Read before the American Association of Anatomists, fifty-fifth annual session, Harvard Medical School, Boston, Massachusetts, April 8, 1939.

has built up some sort of rationale for experimental work in the therapeutic control of menstrual disorders (11). More certain procedures looking towards further alleviation of sufferings peculiar to womankind awaits the continued cooperation of the gynecologist and the student of primate anatomy and physiology.

Irregularity of the Menstrual Cycle

Since it was possible, indeed necessary, to keep very accurate records of the dates of the menstrual flow of monkeys which were being assembled at various laboratories, it soon became apparent that in cycle length the female monkey is anything but regular. This is readily apparent from the typical distribution curve shown in figure 1, A.

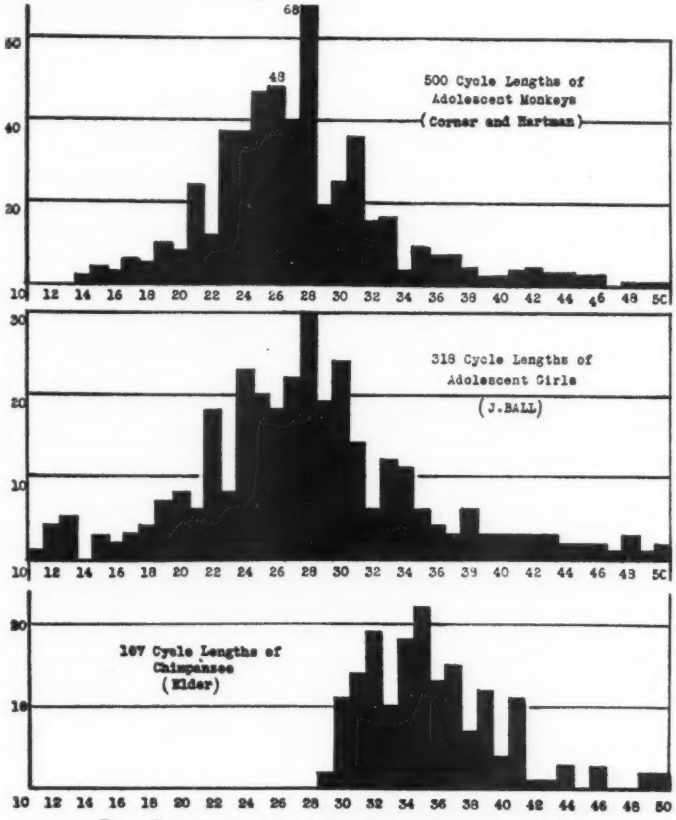


Fig. 1. Distribution curves of length of menstrual cycles for rhesus monkey, young women and chimpanzee.

The first accurate, fairly comprehensive series of observations on cycle length in women was that of King (12), a study inspired by Dr. Corner. Similar contributions have multiplied, as may be seen by a perusal of Arey's recent paper (13), in which all the published, more reliable data have been collected and subjected to further analysis. The graph (14) by Ball shown in

figure 1, *B*, which concerns the experience of young girls, indicates that the human species and the rhesus monkey behave in much the same way with respect to length of the menstrual cycle, for curves *A* and *B*, figure 1, are practically interchangeable. The distribution curves of older monkeys and older women show less spread, that is older females tend to be more regular; but the principle illustrated by the graphs presented is essentially correct.

As emphasized by Arey, the spread of such graphs describes the experience of entire groups; individual females, though never perfectly regular over long periods of time, may be very regular when compared with the whole group. In view of the recently revived interest in the 'safe period' (15) it is the performance of each individual, of course, which is of practical significance. Reliable, long-time menstrual records of women who kept menstrual calendars are now appearing in the literature from time to time (Arey promises to report a number in the near future) and should prove useful. One of the more recent graphs of 140 cycles (16) is presented in figure 2 (left). The deviation from the mode in this case is only 3 days in either direction (if one except 7 cycles) or about the average human experience according to Arey's analysis. For purpose of comparison, 4 cases from the protocols of the Carnegie monkey colony are given in figure 2 (right), as follows.

Monkey No. 2 was received as multipara Oct. 15, 1925 and died of tuberculosis June 22, 1936. She had 7 babies, one delivered by caesarean section. Since a large proportion of her 11 years in the colony was spent in pregnancy and lactation, only 33 cycles were recorded.

Monkey No. 17 was received as pre-adolescent Sept. 10, 1926; killed March 10, 1938. She was castrated Feb. 8, 1932 and used in sex behavior experiments for 6 years. She furnished one baby and two embryos.

Monkey No. 71 was large and fully mature when received Sept. 12, 1928. She was castrated April 20, 1934 and killed Nov. 22, 1937. She had one abortion, furnished the first fertilized monkey ovum; had one baby; yielded one young embryo. She was irregular as to menstrual intervals.

Monkey No. 48 was a large, fully mature female, regular, sterile.

The answer to the question as to the relative 'regularity' of captive rhesus females as compared with women cannot yet be given with finality; but five or six thousand accurately recorded cycles are available in the Carnegie colony and are to be analyzed at a later date. One gets the impression that monkeys are more unpredictable with reference to the onset of the menstrual flow than women, even though the monkey data from the more favorable season of the year, September to April, are used.

That the chimpanzee will fall in line with these conclusions appears certain from a preliminary report of the Yale Laboratories of Primate Biology, which furnished the data on which curve C, figure 1, is based. The chimpanzee cycle averages 5 weeks instead of 4, a fact which should allay any suspicion of lunar influence on the menstrual process (17). In both monkey and man it is a remarkable fact, however, that the average of all cycle lengths tends to approach the lunar month, both for the group and for the majority of individuals, no matter how great the deviation of individual cycles.

At the present time, it is becoming quite generally accepted that the principle of physiological variability holds for the menstrual functions as well as for any other physiological phenomenon. Fränkel was right when he

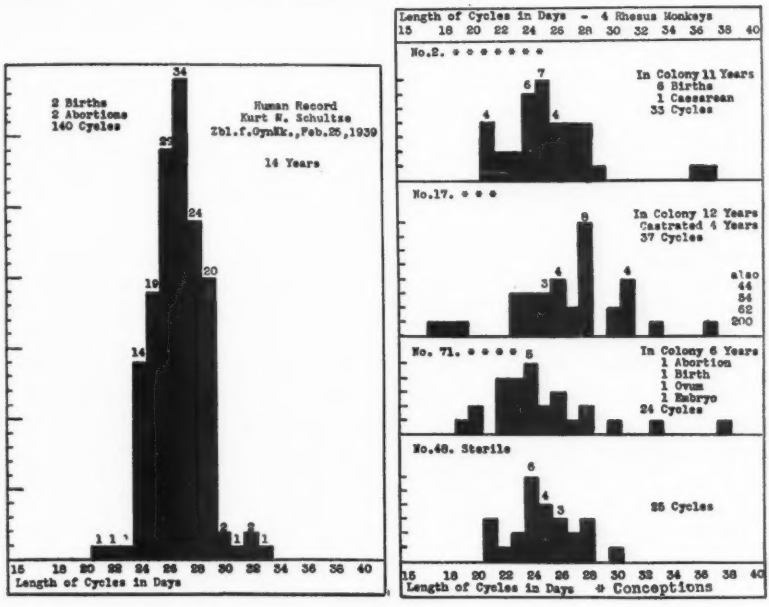


Fig. 2. Graphs of cycle lengths for one woman (after Schultze) and 4 monkeys (Carnegie Colony).

said, with a play on words: "Das einzig Regelmässige an der Regel ist ihre Unregelmässigkeit."

Non-ovulatory Cycles

Heape (18, 19) and Van Herwerden (20) first demonstrated the occurrence of menstruation without ovulation in macaques shot in the wild. Corner in 1923 (3) found examples of the phenomenon in captive rhesus monkeys. Since 1925 the Carnegie rhesus colony has afforded several thousand examples of non-ovulatory menstrual cycles, tabulated in 1937 (21) and presented in table 1.

Table 1. Analysis of non-ovulatory cycles in 300 rhesus females (Carnegie colony)

I. 'Normal' occurrence		No. of Cycles
1. In non-breeding season (May–Sept.)		1,000
2. In adolescence		240
3. In the pre-climacterium.		
4. During recovery from pregnancy and lactation.		
II. 'Pathological' occurrence	No. of Animals	No. of Cycles
1. Palpably sick; amenorrheic	50	
2. Two or more cycles before death	43	200
3. Apparently healthy, never ovulating	11	175
4. Occasional (rare) ovulations	18	175
5. 'Fair' quality but unpredictable	11	100
6. Recently acquired, not yet acclimated	31	150
7. Refractory 2 years; recovery	4	40
8. Refractory 1 year; recovery	15	90
9. Recovery during first year	20	45
10. Good ovulators from start	57	100
Total	260	1,075

In the monkey non-ovulatory cycles are the rule in the summer months, as the writer has repeatedly pointed out (21a); hence menstruation without ovulation is considered 'normal' at that season. Before reaching normal endocrine balance after pregnancy and lactation monkeys frequently menstruate several to many times before resuming the 'complete' cycle which, of course, includes ovulation. The resumption of normality may be gradual, the ovaries and uterus with each cycle showing improvement over the preceding one.

Female No. 217 is of especial interest in that, although as splendid a rhesus female, judged by general activity and appearance, as the Carnegie laboratory ever possessed, she never ovulated during the 4 years in which she was carefully studied by palpation as well as by a dozen laparotomies. She was also very regular, cycles varying between 24 and 27 days in length.

This 'staircase' phenomenon (22) is an outstanding characteristic of the adolescent organism. Here, too, the menstrual function precedes that of reproductive capacity (23). In the Carnegie colony a number of rhesus monkeys have been reared from conception to maturity (several grandmothers have been counted in the colony's roll call) and observations made on first menses and first ovulation. The following is a case in point (+ = ovulatory cycle, o = non-ovulatory cycle). Cycles, in which there was uncertainty as to the occurrence of ovulation, are not included.

Monkey No. 73

Born to No. 2, April 19, 1929
First menses, May 2, 1932 (aged 3 years) 12 cycles without ovulation
First ovulation Jan. 15–17, 1934 (aged 3 3/4 years)
4 further non-ovulatory cycles

1933–4. Oct. 2 to May 31:	o	+	+	+	+	+	o	o	o
1934–5. Sept. 10 to May 20:	o	+	+	+	?	?	o	o	
1935–6. Sept. 16 to May 25:	+	+	+	+	+	+	?	o	
1936–7. Sept. 18 to May 20:	+	+	+	+	+	+	o		

That the human female is by and large, likewise protected in this way against the possibility of too early pregnancy has been demonstrated by v. Mikulicz-Radecki and Kausch (24). That non-ovulatory cycles will also be found very common in women and failure of the ovary to release ova a common cause of sterility, appears certain from studies now appearing in gynecological literature. These studies, stimulated by controversies as to what constitutes 'true' menstruation, have been greatly facilitated by the perfection of the technic of uterine biopsy, which has now become an office procedure. As a general rule the recovery of an interval endometrium a week before the expected menses is regarded as proof of failure of ovulation.

After having diagnosed such a condition, the next step is to do something about it, therapeutically. Davis and Koff (25) undertook the first systematic series of experiments on women with a view to inducing or precipitating ovulation. The writer (26) attempted, with equivocal success, to 'cure' deficient members of the Carnegie rhesus colony and bring about ovulation in response to gonadotropic hormones. The method holds out a modicum of promise to childless couples desperately desirous of bearing offspring.

Time of Ovulation in the Menstrual Cycle

The Ogino-Knaus theory, on the basis of which calculations of the fertile days of the menstrual cycle are commonly based, holds that ovulation occurs 14 to 16 days in advance of the expected menses. This presupposes a constancy in the functional life of the corpus luteum of ovulation (27). Both Ogino and Knaus write somewhat as advocates in defense of the theory. Hence they are more or less justly criticized by gynecologists who, on unscientific because unprovable grounds (chiefly verbal reports of their patients) find the 'safe period' method of birth control frequently fails. The writer (14) has reviewed the subject (1936) and come to the conclusion that the opponents of the Ogino-Knaus theory have yet to bring a single proved case to invalidate the soundness of the theory.

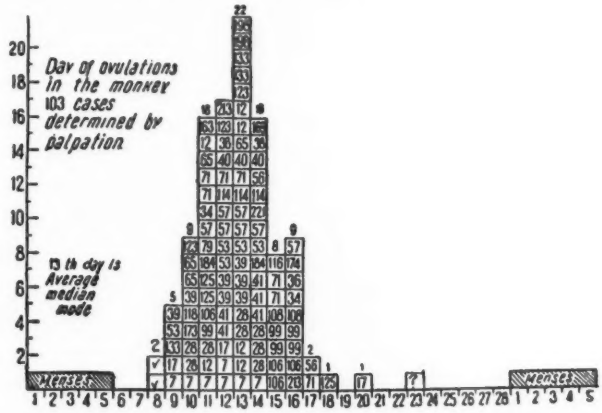


Fig. 3. DISTRIBUTION CURVE OF DAYS OF THE MENSTRUAL CYCLE IN WHICH OVULATION OCCURRED IN THE RHESUS MONKEY

Before Ogino or Knaus, Corner (3) and Allen (4) recovered unfertilized ova from the rhesus monkey, all near the middle of the cycle. Later Pratt, Allen, Newell and Bland (28) accomplished the same crucial verification of ovulation in women. To date monkeys in the Carnegie colony have furnished data on the day of conception in over 400 cases. More than 100 eggs and embryos have been recovered as corroborative evidence; and the seriation of these according to stage of development agrees perfectly with their chronological age as calculated from time of ovulation. This was usually determined by palpation, occasionally by isolated coitus, often by both. Figure 3 represents a sample distribution curve of ovulation on days of the cycle (14). The work of compiling all of the accumulated data is in progress. To date no ovulation has been encountered outside the range of day 8 to 16, except one each on days 17, 19, 20, 21, a doubtful case on day 24, and an experimental ovulation on day 29 (29).

We shall await similar unequivocal data for the human species. While ovulation may not be detected by palpation, the beautiful work of Hertig and Rock, reported at this meeting (30), has demonstrated the feasibility of recovering human embryos by the simple device of scheduling unavoidable hysterectomies according to the fertile period, as worked out on the monkey. With embryological history thus made, first by the Allen-Pratt group (28) and now by the Harvard group (30), we may perhaps look to revival of interest in this direction and the gradual acquisition of a collection of human embryos paralleling the monkey series now in possession of the Carnegie Laboratory of Embryology (31).

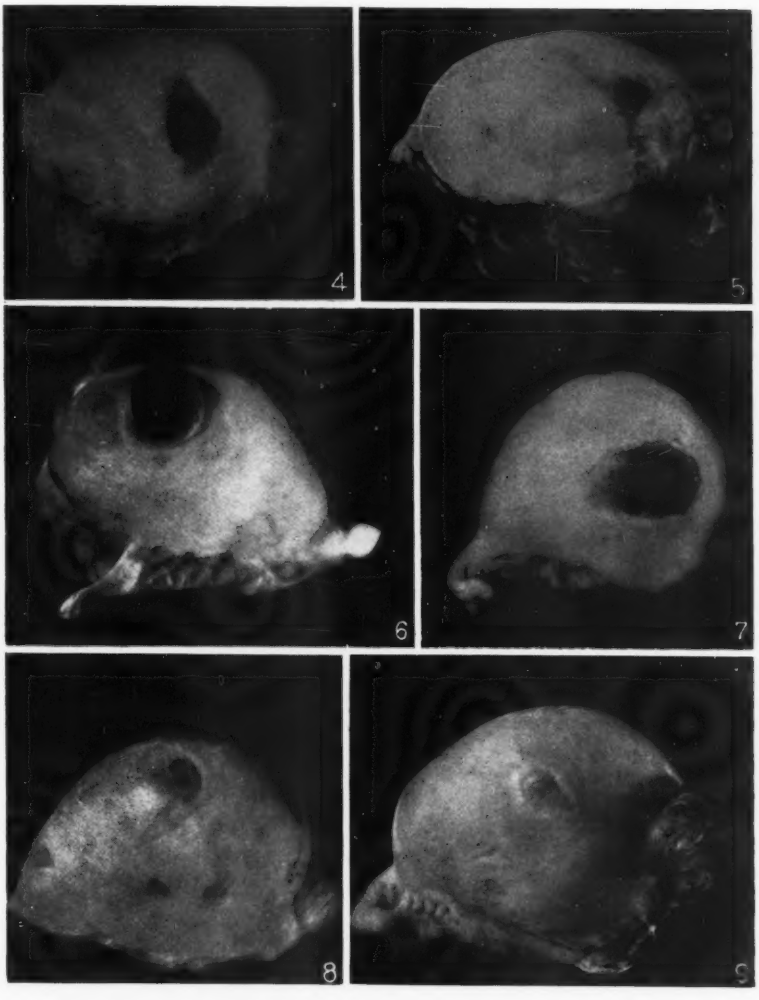
In connection with the problem of the time of ovulation some remarks concerning the estimation of age of corpora lutea on the basis of gross inspection at laparotomy are pertinent. By and large, a very fresh ovulation point or stigma possesses distinguishing characteristics that enable one to be reasonably certain that a 'very recent' ovulation has taken place. Chief criteria are: orifice of ruptured follicle sometimes plugged by very viscous liquor folliculi, which may protrude or even elongate into a ribbon; walls suffused with blood, herniating to a variable extent, but always bright red. However, after healing has been effected, which occurs in a few days, the uncertainty of age estimation increases rapidly with time. To illustrate how easily one can be mistaken, the writer on one occasion, not having looked up the palpation record on a certain animal, on the tenth day after ovulation removed the wrong ovary. In this case a corpus luteum of 40 days happened to look fresher (redder) and more protruding than the corpus luteum of ovulation which was only 10 days old. In figures 4 to 9 are included several very young stigmata, easily recognized: in figures 4 and 5 may be seen 2 different stigmata exactly 11 days old; in figures 6 and 7 two stigmata of like appearance, one 66, the other 2 days old; likewise in figures 8 and 9 two stigmata, respectively 146 and 18 days old, have much in common.

After the first flush of the granulosa of the freshly ruptured follicle, due to a variable amount of hemorrhage, at least in the monkey, perfectly reliable criteria of age, on the basis of direct observation at laparotomy, are thus far lacking. Histological criteria are now being worked out.

II. GENERALIZATIONS OF INTEREST TO GYNECOLOGY AND ANTHROPOLOGY

Thus far emphasis has been placed on facts common to both primate animal and man. Such common denominators argue for the close affinity between man, anthropoid apes and monkeys. These share, for example, the process of menstruation which at the same time differentiates them from all other mammals, as has been already pointed out. However, other facts have come to light from the study of reproductive process, indicative of instructive differences among the primate groups. Because of these very differences the facts presented are of interest to the anthropologist and to the gynecologist alike, lending, as they do, added proof of blood relationships.

Physical anthropology, as the name implies, has been built up primarily on a morphological basis. Number, size, shape and arrangement of teeth;



Figs. 4–9. STIGMATA OR 'OVULATION POINTS' OF MONKEY OVARIES AT VARIOUS INTERVALS FOLLOWING OVULATION, TO ILLUSTRATE UNRELIABILITY OF ESTIMATES OF OVULATION TIME BY INSPECTION OF OVARIES AT LAPAROTOMY. EXTREME EXAMPLES WERE SELECTED. FIG. 4 AND 5, EACH 11 DAYS; FIG. 5 AND 6, 66 AND 2 DAYS, RESPECTIVELY; FIG. 8 AND 9, 146 AND 18 DAYS, RESPECTIVELY.

conformation of jaw, of skull, pelvis and brain—these are morphological concepts. To date, man's place in nature has been based largely on a study of the hard parts since these are most readily preserved and accurately measured. Nevertheless, though exact, the results from the standpoint of primate relationships have been far from harmonious. Seriation of the primates with respect to one character turns out to be quite different with respect to other characters. This holds true not only for teeth and skeleton, but

quite generally for non-skeletal structures such as muscles and viscera as well.

On the whole, the preponderance of morphological evidence favors the man-in-the-street idea of the alignment of the primate orders, namely:

Man
↓
Anthropoid apes (chimpanzee, gorilla, orangutan)
↓
Hylobatidæ (gibbon, siamang)
↓
Old World Monkeys or Catarrhines (macaques, baboons, etc.)
↓
New World Monkeys or Platyrrhines (spider, howler, capuchin, marmoset, etc.)

Some characters, as the brain, for example, have given clear-cut data. There is no doubt that in size, shape, contour and cytoarchitecture the

Fig. 10. TEN AND ONE-HALF-DAY CHIMPANZEE OVUM (right) AND 11- OR 12-DAY HUMAN OVUM (the 'Miller' ovum) TO SHOW SIMILARITY IN MECHANISM OF EARLY IMPLANTATION.

anthropoid brain approaches more closely to the human than to the monkey brain. Furthermore, so far as investigated, a true Babinsky reflex, which shows up clinically in man, has been produced experimentally only in the chimpanzee, not in the monkey. Among physiological characters, specific blood-group agglutinogens point to almost perfect identity of chimpanzee blood with that of man and to distinctions between the groups just mentioned, while within each group a degree of similarity obtains.

Of just such an order of definiteness are the following items which have emerged in recent years from certain cooperative studies in reproduction in the rhesus monkey, the chimpanzee and man: (*a*) the characteristics of the earliest implantation of the ovum and the reaction of the maternal tissue to the implanting ovum; (*b*) the content of the urine of pregancy in gonadotropic hormones; (*c*) the intra-uterine growth rate, period of gestation and maturity of the newborn.

Implantation of the Ovum

Interstitial vs. Shallow implantation. The youngest human ova, now numbering three, 12 days old or younger, the 'Miller' (32), the Hertig-Rock *A* and *B* (30), had already penetrated the uterine epithelium and burrowed into the stroma. It is evident, therefore, that in man, implantation is interstitial.

In the monkey, on the contrary, the ovum which is also of the burrowing type, remains superficial (31). Indeed, it gradually so affects the opposite surface that a secondary placenta forms there and a double discoidal placenta is the result. Therefore the embryo within its envelope remains suspended, as it were, between the dorsal and ventral walls of the uterus.

Such double placentæ characterize all known cases of Catarrhines and most of the Platyrrhines. In those Platyrrhines that have simple placentæ the secondary site shows considerable reaction, only to atrophy and disappear. Even in one of the human specimens Hertig has noted a slight reaction on the endometrial wall opposite the embryo. For it should not be forgotten that the uterine cavity is but a potential one, the surfaces touching except for a film of 'uterine milk.'

What then is the condition in the anthropoid apes? Older specimens recovered from the orangutan and the gibbon contained a decidua capsularis, an area above the embryo where villi are sparsely developed (33). This taught us to expect an interstitial implantation in the anthropoids.

The matter was definitely settled by the fortunate recovery of a 10½-day chimpanzee ovum, 'Yerkes *A*' (34) through cooperative efforts of the Yale Laboratory of Primate Biology at Orange Park, Florida, and the Carnegie Laboratory of Embryology at Baltimore. As seen in figure 10 the ovum had already sunk beneath the surface of the uterine lining. It is very similar to the two new ova from Harvard which are just a shade older. The identity in method of implantation between man and ape is, therefore, practically perfect. Both differ strikingly from all the monkeys.

The placental plaques. In rodents, and to some extent in man and the chimpanzee, the pregravid uterus sensitized by the corpus luteum hormone, presents the 'decidual reaction' in response to mechanical or chemical stimuli, e.g. to the ovum. The decidual cells are stromal, being modified connective tissue cells. In the monkey, on the contrary, it is the uterine epithelium which responds. The presence of the ovum causes a proliferation of the epithelium within a considerable range, both on the surface where it will first imbed (primary placenta), usually on the ventral side, as well as on the opposite surface where the secondary placenta will form. The proliferation is intense and the epithelial cells pile up into a thick plaque. These, for about 15 days, become a prey to the destructive and phagocytic action of the invading trophoblast. Such plaques may be produced artificially under proper hormonal influence (35).

All monkeys as well as Tarsius, a lemmoid, develop placental plaques in the earliest stages of implantation (33), and such plaques are unknown among the anthropoids and man. The issue is clear-cut; no transitional conditions are known to occur.

Prolan in the Urine of Pregnancy

After the discovery of the presence of anterior pituitary-like substances in the urine of pregnant women the search was made for its occurrence in other mammalian species. Results of tests in all the lower mammals were negative, but in the chimpanzee Zuckerman (36) as well as Schultz and Snyder (37) demonstrated the presence of gonadotropic material, at least during the first 100 days of gestation. By more systematic application of the Friedman test throughout pregnancy, Elder (personal communication) determined the first positive reaction between days 25 and 35, its disappearance sometime between days 100 and 130 of gestation.

Testing pregnant monkeys of the Carnegie colony from the 30th day of gestation on, Snyder and Wislocki (38) obtained only negative results.

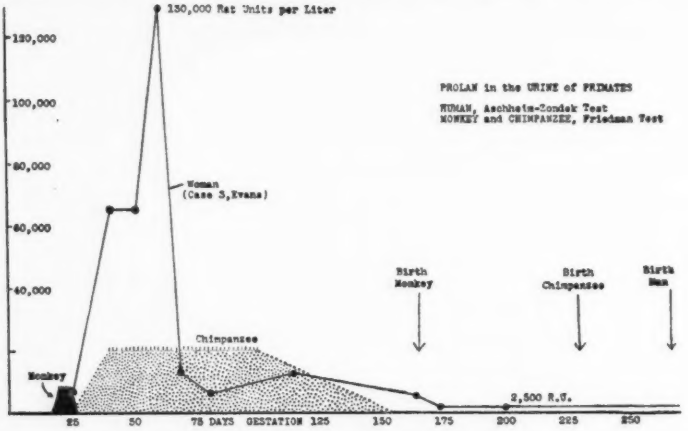


Fig. 11. The appearance of prolan (APL) in the urine of pregnant monkeys, chimpanzees and women.

Hamlett (39), however, thinking phylogenetically on the problem, considered it worth while to test monkey urine of pregnancy before the 30th day, with the result that he discovered the presence of prolan for one week only, namely from the 18th to the 25th day of gestation.

A word might be added concerning urinary pregnandiol in human pregnancies, a finding of growing importance to the obstetrician. Astwood and Weil (unpublished) were both unable to isolate any of this material from the urine of our pregnant macaques. From a further study which Dr. Marker is now making on the sterols in the pregnant monkey's urine it seems that this contains no pregnandiol of any kind. Chimpanzee urine has not yet been assayed for pregnandiol.

We have then a phylogenetic series. In man, prolan is found in the urine throughout gestation, with a brief but enormous increase around the 60th day (40, 41); in the chimpanzee a positive Friedman test is seen only in the second and third and part of the fourth month of the 8½ months gestation;

in the monkey prolan appears in the urine for only a week, disappearing about the time that the placental plaques have disappeared. These facts are illustrated in figure 11.

Intra-uterine Growth

We are indebted to Streeter and to Schultz for the growth curve in man, to Schultz for that of the rhesus monkey and the fragmentary data on the chimpanzee. The three curves are shown in figure 12. The curve for man is smoothed out because it is based on the averages of a sufficient number of cases. The curve for the rhesus monkey includes not very many cases, but these were accurately timed with respect to day of conception (Carnegie Colony cases) and cover the entire period of gestation. The curve for the chimpanzee is strictly tentative because based on too few cases, mostly confined to the last third of gestation. The chimpanzee growth curve will be more precisely analyzed by Schultz (42) and two new cases added.

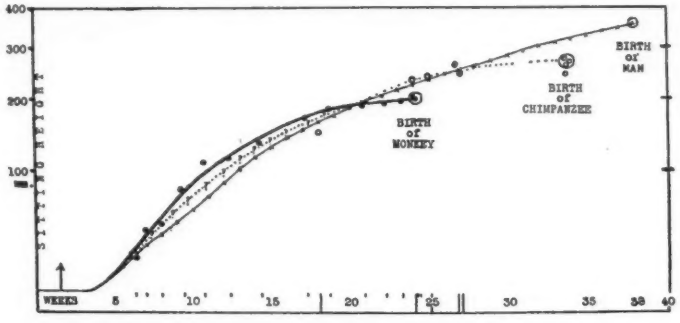


Fig. 12. Prenatal growth curves for monkey, chimpanzee and man.

Certain points of interest to the present paper appear on inspection of the curves.

(*a*) Until about the 30th day man and monkey have about the same growth rate.

(*b*) After the first month the monkey embryo grows somewhat faster than the human embryo.

(*c*) At about the 20th week man, monkey and probably also the chimpanzee are of about the same size. When this phenomenon is considered in connection with the fact that the definitive placentæ of man, ape and monkey are practically identical, we may say, in the words of Dr. Streeter, that it appears from this that a given mass of placenta is capable of supporting a given bulk of baby.

(*d*) In respect to the length of gestation the chimpanzee (8½ lunar months) is intermediate between that of monkey (6 lunar months) and man (10 lunar months).

(*e*) The monkey baby at birth is relatively mature, being capable of holding on to the mother at the instant of birth. The chimpanzee baby is more like the human in helplessness at birth.

SUMMARY

In the preceding survey of recent work on the physiology of reproduction in man and his nearest kin, the apes and monkeys, an inventory was made of a few of the newer and more significant facts likely to influence our thinking with respect to man himself. Monkeys and apes, it is seen, serve to bridge the gap in an important way between the lower mammals, in which most of the fundamental researches must of necessity be made, and man himself.

Permission to reprint from *The American Naturalist* figures 1, 10, 11, 12 is gratefully acknowledged.

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A COMPARISON OF FOUR METHODS OF BIOASSAY FOR THE GONADOTROPIC FACTORS

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THE OBJECT of a gonadotropic assay is to evaluate the amount and kind of gonadotropic stimulation produced by a given extract, whether derived from the gland, blood or urine.

The factor or factors producing such stimulation cause follicle growth and luteinization in the ovaries. Follicle stimulation is followed by production of estrogen which, in rodents, causes proliferative changes in the uterine mucosa and breasts as well as mucification of the vaginal epithelium. Luteinization (corpus luteum formation) produces progesterone which induces secretory changes in the estrogen-stimulated uterine and mammary epithelium.

The gonadotropic effect may be evaluated directly from the ovaries or indirectly by evaluation of the ovarian action initiated by the gonadotropic factors, as manifested by the secondary effects produced on the rest of the genital system (uterus, vagina, breasts).

Gonadotropic extracts have never been purified. The probability therefore exists that varying and incalculable mixtures of follicle stimulating and luteinizing factors are present in each precipitate that is tested. These differences are most apparent in contrasting the urine from different groups of women, such as cyclical, amenorrhoic, castrate and pregnant.

Further differences arise from the method used in concentrating the factors. It appears that the benzoic and tannic acid methods, for example, concentrate mainly or exclusively the follicle stimulating; the acetone and acid alcohol methods, on the other hand, concentrate both follicle stimulating and luteinizing elements.

We have shown (1, 2, 3) that not only follicle stimulation, but also luteinization may be obtained with the urine of cyclical, amenorrheic and menopause patients. To be satisfied with demonstration of the follicle stimulating factor alone appears insufficient, as this gives an incomplete and one-sided picture of hormonal conditions. For this reason, we employ the acid alcohol method for urine concentration which concentrates both factors (4).

What follows is an effort to determine which method of bioassay (contrasting various technics as used on the immature rat) affords the most satisfactory picture of the stimulation exerted upon the ovaries and genital tract of the test animal.

The present study was undertaken as a preliminary investigation of the comparative merits of four methods, namely, (*a*) the premature vaginal opening; (*b*) the ovarian weight; (*c*) the uterine weight; (*d*) the ovarian values (morphology). The unit used for each of these methods differs widely. (*a*) For vaginal opening, some investigators use three criteria; vulvar swelling, vaginal opening, vaginal opening with positive smear (5); others note vaginal opening alone. (*b*) Ovarian weight, the minimum amount of precipitate which produces an increase of a 50 to 100% above the weight of untreated controls (6). (*c*) Uterine weight, the minimum amount of precipitate which increases uterine weight by 100 to 150% above that of the untreated controls (7). (*d*) Ovarian values based upon serially sectioned ovaries in which numbers from 1 to 9 are assigned to various degrees of follicle growth and luteinization. For ovarian values it was found expedient to define two units, the first being the amount of precipitate which produces an enlargement of 6 follicles; the second, the amount of precipitate which causes the entire ovary to be occupied by contiguous corpora lutea.

In the experiments here reported, all urines were concentrated by the modified Zondek (acid alcohol) method recently described (4). The resultant dry powder, kept in a desiccator, was dissolved in distilled water as required. The total dose, subdivided into 5 equal portions, was injected subcutaneously twice daily (morning and afternoon) distributed over 2½ days. The animals were killed 96 to 100 hours after the first injection, by illuminating gas and autopsied at once. The extracts (whether derived from castrate or pregnancy urine) in small dosage produced follicle stimulation, and in increased dosage luteinization. The animals used were immature albino rats, of the same strain employed for the past 7 years. At the beginning of the experiment they were 26 to 28 days old and weighed 30 gm.

At autopsy open or closed vagina was noted.

The ovaries were stripped of tube and bursa, then the combined weight of the two ovaries recorded by means of a torsion balance reading to 1 mg.

The uterus was stripped, emptied, blotted twice if it contained fluid, and then weighed.

The ovaries were fixed in Bouin's fluid, embedded in paraffin, sectioned and studied. Ovarian values were determined on an arbitrary scale, based upon the facts that ascending amounts of extracts produce morphologically recognizable changes in the serial sections of the ovaries as noted below.

0, Control ovaries
1, Very slight increase of follicles
2, Slight follicle growth
3, 1 large follicle
4, 3–4 follicles enlarged
5, 6 follicles enlarged and cystic
6, Contiguous follicle enlargement
7, 1–2 corpora lutea
8, 3–4 corpora lutea
9, contiguous corpora lutea[1]

Exp. 1. Urine from surgical castrate, 52 years of age, operated upon 6 years ago

[1] Combined reactions, for example, 3–4 follicles (#4) and 3–4 corpora lutea (#8) in the same ovary were charted as 4+8/2=12/2=#6.

(supravaginal hysterectomy and bilateral oöphorectomy) was used; 139 mg. of powder equalled 100 cc. of urine.

Figure 1 is based on 77 rats, shows ovarian weights, uterine weights and ovarian values. Uterine weight reaches an apparent peak at 40 cc., the 3 others at 80 cc.

Exp. 2. Urine from surgical castrate, 51 years of age; operated upon 7 years ago (supravaginal hysterectomy and bilateral oöphorectomy) was used; 85 mg. of powder equalled 100 cc. of urine.

Figure 2 is based on 104 rats, and shows the 3 bioassay criteria. Uterine weight again reaches a peak at 40 cc. All except ovarian values show apparent recession at 60 cc. All show apparent plateau values bevond 80 cc.

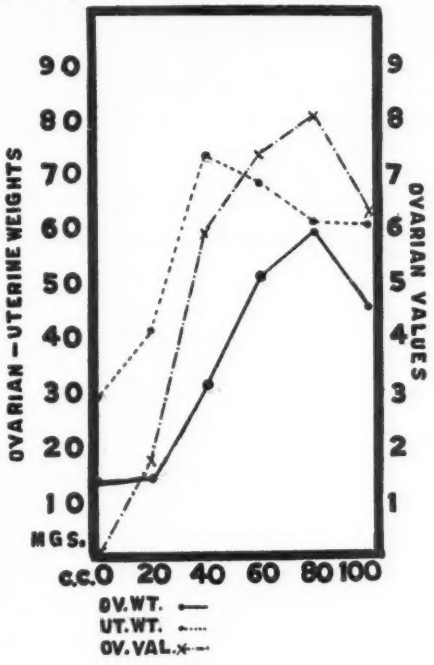

Fig. 1. Female castrate urine.

Exp. 3. Urine from two pooled pregnancies of 8 and 9 weeks was used. 147.6 mg. of powder equals 100 cc. of urine.

Figure 3 is based on 135 rats, and shows that ovarian weights fail to show an evaluable increase. Uterine weight and ovarian values show progression.

The uterine weight increase is steep between 0 and 0.05 cc.; the ovarian value shows progressive increase between 0 to 0.3 cc.

DISCUSSION

The results obtained with extracts of the urines of two surgical castrates and one pregnancy urine, all concentrated by the acid alcohol method, contrast the curves obtained in these different experiments. They do not warrant broad generalizations but serve as a guide for future study.

Vaginal opening as an index of gonadotropic effect cannot be used for pregnancy urine (no progressive increase). Moreover, observation on 1120 rats, not included in this series, has shown that vaginal opening occurs when the ovaries, judged by morphological changes, are negative and that the vagina remains closed when the ovaries are strongly positive. Vaginal opening never occurred in control animals. However, all efforts to correlate opening with either the follicle stimulating or luteinizing factor failed completely. That the opening cannot be ascribed to estrogenic contamination of the extracts is shown by the fact that precipitate equivalent to 100 cc. of pregnancy urine (125 times the largest, 2000 times the smallest amount producing vaginal opening) failed to show estrogenic reaction in castrated mice.

Ovarian weight curves followed uterine weight and ovarian value curves in castrate urine. With pregnancy urine no significant progressive increase in weight is noted.

Uterine weight curves all appear to rise to a peak and then drop by a significant amount to a value which may or may not represent a plateau

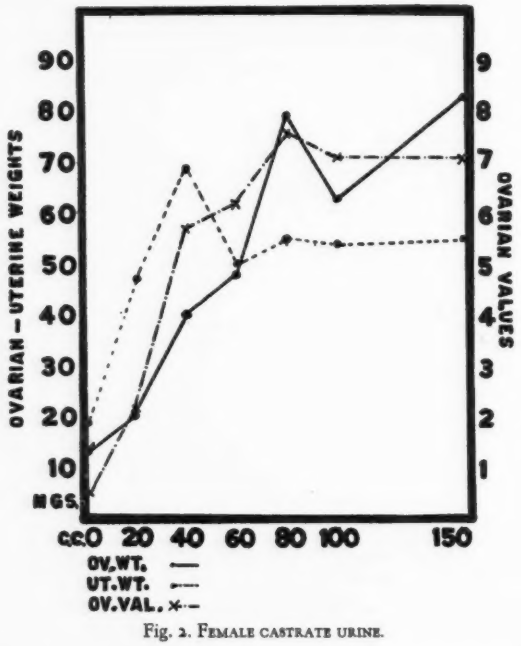

Fig. 2. Female castrate urine.

value. In the two menopause experiments the plateau value may be the same (63.4 and 54.3) but in the pregnancy experiment the value is significantly higher (84.5).

The ovarian values register directly the gonadotropic effects. This method entails far more technical work and also introduces a subjective

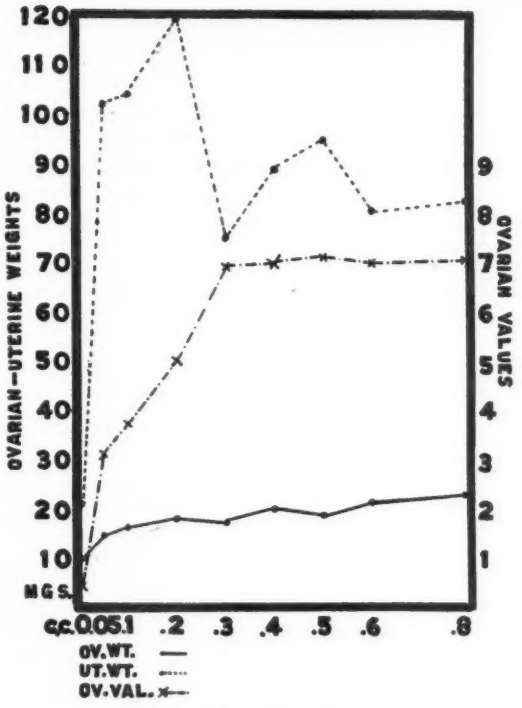

FIG. 3. PREGNANCY URINE.

factor in the reading of the slides, a factor which can be reduced to a minimum by experience and care.

SUMMARY

None of the methods of bioassay of the gonadotropic factor or factors are better than roughly quantitative.

The follicle stimulating factor is concentrated by all the methods currently employed.

Luteinization effect is lost or greatly diminished except by the acetone or acid alcohol method of concentration. Therefore a more complete evaluation of conditions present is obtained by the acid alcohol method of concentration.

Vaginal opening appears unreliable for bioassay. Further investigation is needed to determine the forces which produce this phenomenon.

Ovarian weight increase indicates the amount of follicle stimulating factor present. It does not show the luteinizing factor, most evident when the urine of pregnancy is assayed.

Uterine weight increase indicates the amount of follicle stimulating factor present but does not permit of evaluating the luteinizing factor.

A complete bioassay of the gonadotropic factors necessitates: (*a*) a concentration method which precipitates both follicle stimulating and luteinizing factors (acetone, acid alcohol methods), (*b*) ovarian 'values.' The follow-

ing is suggested as the units: (*a*) the smallest amount of material which produces the full growth of 6 follicles, (*b*) the smallest amount of material which produces contiguous corpora lutea.

Comparing the relative merits and disadvantages of these several procedures, we have decided to continue to use the ovarian values as our method for bioassay, because it appears to present a more comprehensive picture of the gonadotropic forces at work. We realize, however, that a more extensive statistical investigation of this problem is required in order to justify this choice for general adoption.

We desire to express our appreciation of the valuable help given by Dr. Franklin Hollander in developing an appropriate scale of ovarian values.

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EVIDENCE OF HYPERFUNCTION OF THE ANTERIOR PITUITARY IN A STRAIN OF RATS[1]

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AN ANALYSIS of the factors possibly responsible for the low glucose tolerance of the Yale strain of *Mus norvegicus albinus* as compared with that of the Wistar strain early directed the attention of the authors to the rôle of the anterior pituitary (1). The striking difference between the growth rates of the two strains maintained on the same diet and under the same conditions associated with a higher fasting blood sugar in the more rapidly growing Yale strain suggested a comparison of the two strains on the basis of other phenomena associated with a hyperactivity or unbalanced activity of the pars anterior. In the present communication the two strains have been compared using as the criteria: (*a*) epinephrine tolerance, (*b*) insulin tolerance, (*c*) water balance, (*d*) sterility, (*e*) growth and (*f*) body fat.

EXPERIMENTAL METHODS

Rats. The authors' colony of the Yale strain was started in the fall of 1935 and that of the Wistar strain in the summer of 1937. Since the latter date the two colonies have been maintained on the same diet[2] and under the same conditions. There has been no Bartonella or other parasitical infection in either colony since January 1937. Although the growth curve of the Yale strain closely approximates that of the parent colony (2) our practice of mating animals from the lowest glucose tolerance range has intensified this trait and possibly has accentuated other tendencies. The Wistar strain has retained the characteristics of the parent colony. Only male rats, paired according to age from the two strains, were used. No anesthetics were employed.

Blood sugar was determined on 0.2 cc. of blood, by the Shaffer-Hartmann-Somogyi method[3] (3) using filtrates prepared by the zinc hydroxide precipitation procedure of Somogyi (4).

Urine collections were made from individual cages, 9.2 in. in diameter and 10 in. high, in which the animals had free access to water and moistened food with the containers so arranged that no water and very little food contaminated the urine. Furthermore, the rats could not urinate into the food or water containers. The urine

[1] This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

[2] The stock diet consists of Purina Dog Chow checkers with lettuce twice weekly. For the breeders and rats under 40 days of age the stock diet is supplemented with a mixture consisting of: whole wheat flour, 50 gm.; whole milk powder, 25 gm.; dried yeast, 20 gm.; cod liver oil, 4 gm. and sodium chloride, 1 gm.

[3] Reagent No. 50 containing 1 gm. of potassium iodide per liter.

dropped through the false bottom of the cage on to a paraffin coated metal funnel, 9.5 in. in diameter and 2.7 in. from the base to the tip of the cone. Fecal masses were held on the funnel by a trap over the spout.

From the funnel the urine was collected under oil in a graduated cylinder. The space between the top of the graduated cylinder and the bottom of the funnel was sealed with a cellophane cylinder. During the series of experiments the temperature of the room ranged from 18° to 24° C. but during a single collection period the fluctuation was much less. With a few exceptions, the relative humidity was 30 ± 5. Urine from the rat pair representing the two strains was always collected during the same period. Before placing the rats in the individual metabolism cages a firm massage of the abdomen over the bladder produced micturition in the course of 3 to 5 minutes and before removing the rat from the cage the abdomen was again massaged. Urine was collected over a 24-hour period and the time was accurate to within 15 minutes.

Total body fat was determined by the saponification method of Leathes and Raper (5) modified as follows. After killing the rat with illuminating gas the intestinal contents were discarded and the total carcass was placed immediately in 30% potassium hydroxide (1500 cc. per kg.) and digested on a steam bath. Ninety-five per cent alcohol was added at intervals to hasten saponification and when no appreciable amount of fat remained visible (10 to 12 hours heating was required) the digest was transferred quantitatively to a graduated flask. The bone residue was heated with 50 cc. of 30% potassium hydroxide, extracted 3 times with hot 95% alcohol, and the alcohol and water-soluble material together with some fine bone suspension transferred to the graduated flask and diluted to volume with alcohol and water. The final alcohol concentration was 25%. To complete the saponification a 50 cc. aliquot was transferred to a 400 cc. erlenmeyer flask and 10 cc. of absolute alcohol, plus solid KOH sufficient to make a final concentration of 20%, were added. The solution was refluxed in a boiling water bath for 4 hours, after which the saponified material was neutralized to litmus with concentrated HCl and extracted with petroleum ether. The ether was evaporated, the residue dried for 16 hours at 65–70° C. and weighed.

RESULTS

Epinephrine tolerance. In 1934 Cope and Marks (6) observed that the injection of saline extracts of the anterior pituitary produced in rabbits a marked sensitivity to subcutaneously administered epinephrine. The injection of 0.2 mg. of epinephrine hydrochloride per rabbit produced in the anterior pituitary-treated animals a hyperglycemia with an area under the blood sugar curve 65% greater than that of the untreated animals. Young (7) confirmed the observations of Cope and Marks and using fractionated preparations of the anterior pituitary demonstrated that the effect was produced by a factor not identical with prolactin, thyrotropic hormone, gonadotropic hormone, or the oxytocic or the vasopressor substances. He suggested the term 'glycotropic substance' for the new factor.

By comparing the results of Cope and Marks and Young with the data shown in figure 1, it is apparent that the difference between the response of the Yale and Wistar strains to subcutaneously injected epinephrine highly suggests a greater activity of the 'glycotropic anterior pituitary substance' in the Yale strain. Subsequent to the injection of 0.2 mg. of epinephrine hydrochloride per kg., the hyperglycemic area produced in the Yale strain is 75% greater than that produced in the Wistar strain and the shape of

the area representing the difference between the response of the strains is essentially identical with that obtained by Cope and Marks comparing normal and anterior pituitary-injected rabbits. A statistical analysis of the data used in making the curves indicates that at each of the points shown the chance that the two curves are identical is of the order of 1 per billion. The rat pair from the two strains was injected, using a 5-minute interval, with the same syringe, into the same subcutaneous area, and from the same solution.

Insulin tolerance. Thirty minutes after the intramuscular injection of 1.5 U of insulin per kg. the blood sugar of the Yale strain fell 6 mg.% while that of the Wistar strain fell 24 mg.% (table 1). Statistical analyses indicate that the chance that this difference is not significant is 2 per billion. Subsequent blood sugar values determined at hourly intervals for 5 hours showed

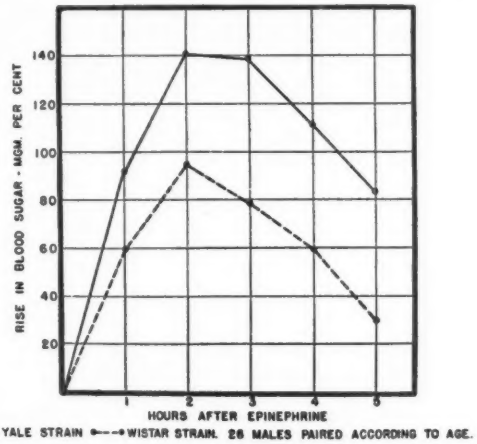

Fig. 1. A COMPARISON OF THE BLOOD SUGAR RESPONSE OF MALE RATS FROM THE YALE AND WISTAR STRAINS FOLLOWING THE SUBCUTANEOUS INJECTION OF 0.2 MG. PER KG. OF EPINEPHRINE HYDROCHLORIDE. AGE RANGE 172 TO 360 DAYS. FOOD WAS WITHDRAWN 15 TO 16 HOURS BEFORE THE INJECTION.

no significant difference between the two strains. Although the insulin antagonism is not as striking as that observed in rabbits treated with anterior pituitary (6, 7), nevertheless, the direction of the results is the same and the quantitative difference may be a species effect.

The authors' results with insulin suggest the possibility of a slower absorption from the intramuscular injection in the Yale strain. However, if the insulin resistance and the epinephrine sensitivity are to be explained on the basis of the rate of absorption, the data require the conclusion that epinephrine is absorbed faster by the Yale strain while insulin is absorbed faster by the Wistar strain. Hence, it appears that a satisfactory explanation of the results will involve factors other than rates of absorption.

Considering the well established hyperglycemic properties of anterior hypophyseal extracts (8–10) the higher fasting blood sugar of the Yale rats

as compared with the Wistar rats (table 1) suggests a higher level of activity of the anterior pituitary in the former strain. The statistical analysis of the means indicates that the difference is significant and a large series of results previously published by the authors (1) showed differences of the same order.

Water balance. The literature on diabetes insipidus recently critically reviewed (11) definitely indicates the presence of a diuretic factor in the anterior pituitary. Support for this point of view has been obtained from a variety of approaches among which may be mentioned: (*a*) the implantation of the pars anterior in hypophysectomized dogs (12); (*b*) the injection of anterior pituitary extracts into hypophysectomized and normal dogs (13)

TABLE 1. A COMPARISON OF THE BLOOD SUGAR RESPONSE OF THE YALE AND WISTAR STRAINS TO THE INTRAMUSCULAR INJECTION OF INSULIN[1]

	No. of rats	Age range	Fasting[2]	Hours after insulin					
				1/2	1	2	3	4	5
			'True' sugar in terms of glucose per 100 cc. of blood						
		days	mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m
Yale	190♂	150–360	72±1.25	66±1.91	37±1.34	29±0.93	40±1.95	53±2.44	61±1.76
Wistar	180♂	155–219	63±0.54	39±0.90	27±0.99	26±0.71	35±1.84	50±1.43	58±1.57
Chance that difference is due to sampling			7/million	1/billion	6/100,000	9/100	21/100	48/100	40/100
			Fall in blood sugar						
				mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m	mg. P.E.m
Yale	190♂			− 6±1.66	−35±1.13	−43±1.22	−32±2.14	−19±2.99	−11±2.11
Wistar	180♂			−24±1.00	−36±0.87	−37±0.60	−28±1.75	−13±1.39	−5±1.34
Chance that difference is due to sampling				2/billion	62/100	3/1000	33/100	21/100	18/100

[1] 1.5 U per kg.
[2] Food withdrawn 15 to 16 hours before control blood.

and (*c*) the difference in the diuretic response of the rat following postlobectomy and hypophysectomy (14, 15).

In a carefully controlled series of experiments, the results of which are recorded in table 2, the authors found that the urine excretion per unit of body weight of the Yale rats conspicuously exceeded that of the Wistar rats and interpreted the data as evidence of a greater activity of the anterior pituitary in the Yale strain.

One hundred and thirty individual male rats from the two strains, paired according to age, were used in these experiments. Some of the animals had been used for glucose tolerance tests 6 weeks prior to the urine collections, the others had been subjected to no experimental procedure and all of the animals appeared to be in good condition at the time of the recorded observations.

Repeated tests showed that the 24-hour volume could be duplicated satisfactorily. Duplicate tests, made 8 to 30 days after the original test on 37 mature rats from the two strains, differed from the original by an average of 0.9%. In the age range 51 to 70 days, representing sexually immature rats, the two strains excrete approximately the same volumes of urine but in the succeeding age groups the percentage of Yale rats in the higher volume brack-

ets always exceeds that of the Wistars (table 2). The summary of the data shows that 20% of the Yale rats excrete more than 4 cc. per 100 gm. of body weight while only 6% of the Wistars belong in this group.

The specific gravity was determined on 60 urines distributed through the various volume groups with averages as follows: volume 1 to 2 cc., sp.

TABLE 2. A COMPARISON OF THE 24-HOUR URINE VOLUMES OF THE YALE AND WISTAR STRAINS[1]

Urine per 100 gm. of body weight	Age in days											
	51–70						71–90					
	No. of rats		Av. wt. of rats		Av. vol. of urine cc./100 gm.		No. of rats		Av. wt. of rats		Av. vol. of urine cc./100 gm.	
cc.	Y	W	Y	W	Y	W	Y	W	Y	W	Y	W
1–2	1	1	360	210	1.89	1.90	2	0	326		1.89	
2–3	1	2	368	181	2.74	2.77	3	5	329	284	2.48	2.59
3–4	2	3	267	213	3.45	3.55	3	0	318		3.19	
4–5	2	2	209	176	4.35	4.20	1	0	289		4.08	
7–8	1	0	228		7.89							
	91–120						121–160					
2–3	5	3	363	292	2.64	2.79	1	6	398	300	2.46	2.53
3–4	5	8	353	294	3.32	3.40	1	3	421	328	3.39	3.49
4–5	3	1	357	297	4.51	4.03	1	1	396	344	4.04	4.36
5–6	1	0	367		5.95							
	161–200						201–240					
1–2	0	2		343		1.91						
2–3	9	12	546	327	2.64	2.44	3	6	555	380	2.75	2.51
3–4	7	6	478	325	3.49	3.31	5	1	584	404	3.36	3.22
4–5	2	0	462		4.23		1	0	630		4.05	
	301–350						Summary					
							Av. urine production for each group		Av. water consumption for each group		Total no. of individual rats in each group	
							Y	W	Y	W	Y	W
1–2	1	1	462	450	1.73	1.89	1.85	1.90	4.24	4.57	4	4
2–3	3	2	555	459	2.43	2.45	2.60	2.53	5.53	5.66	25	36
3–4							3.38	3.40	5.97	7.36	23	21
4–5							4.28	4.20	7.10	7.58	10	4
5–6							5.95		10.55		1	0
6–7												
7–8	1	0	625		7.46		7.68		10.68		2	0

[1] Sixty-five individual males from each strain were used.

gr.—1.0529; volume 2 to 3 cc., sp. gr.—1.0516; volume 3 to 4 cc., sp. gr.—1.0390; volume 4 to 5 cc., sp. gr.—1.0379; volume 5 to 6 cc. (one determination) sp. gr.—1.0197; volume 7 to 8 cc. (one determination) sp. gr.—1.0067. In the same volume group the specific gravity of the Yale strain was slightly less than that of the Wistar strain except in the 1 to 2 cc. range.

The water consumption during a single 24-hour period cannot be measured accurately but the averages, for the 65 animals from each group shown

in table 2, should represent the order of the volumes consumed. These averages show that the increased urine excretion is accompanied by an increased water consumption. The relation between the urine excreted and the water consumed is approximately the same for the two strains in a given volume range and the percentage of the water consumed appearing in the urine varies from 41% in the urine volume range 1 to 2 cc. per 100 gm. to 72% in the range 7 to 8 cc.

Sterility. Although the data at this time are not conclusive, for the past 1 1/2 years the authors' colony of the Yale strain has shown an appreciable incidence of sterility. Attempts to mate animals in the lowest glucose tolerance range have been frustrated frequently by their failure to breed and the examination of the breeding records for this period showed that 27% of a series of 22 Yale males which we attempted to breed were definitely sterile. During the same period an examination of the records of Wistar males showed that all of the males selected for breeders, 15, were fertile. The ages of the two groups were comparable. The high incidence of sterility in human gigantism and acromegaly and also the sterility produced in experimental animals by the injection of extracts of the pars anterior of the ox (16, 17) suggest that the lower fertility in the Yale strain may result from a hyperfunction of the anterior pituitary.

Growth. A comparison of the records from the Yale Laboratories of Physiological Chemistry and the Wistar Institute of Anatomy on the growth of *Mus norvegicus albinus* show that for many years the Yale rats have grown faster and larger than the Wistar rats (2, 18, 19, 20). The Yale investigators have attributed the difference principally to the diet, which in the Yale laboratory has kept pace with the rapid advances in nutrition, but in the authors' laboratory rats from the two strains have been kept on the same diet and under the same conditions for 3 generations or longer with a persistence of the quantitative differences in the growth and size.

Except for the weaning weights and those immediately following, the growth curve of the Yale strain in our laboratory checks well that of the original colony (2) and our growth records on descendants of the Wistar stock check the 1937 report from the Wistar Institute. In attempting to account for the size of the Yale strain a quotation from Smith and Bing (21) is helpful, "These accelerated rates of development have resulted seemingly from improved feeding methods, although the continuous selection of thrifty animals for breeders is a factor to be considered in any interpretation of this phenomenon." The Wistar Institute has avoided the selection of exceptional animals for breeding and the diet, consisting of a wide variety of natural foods, has remained essentially unchanged for many years (20).

It may be important that the difference between the growth rates of the two strains closely parallels the difference produced by the administration of anterior pituitary extracts (fig. 2). In this figure we have plotted the 1938 growth rates of males of the two strains in our laboratory and have added the results obtained (16) following the administration of anterior pituitary extracts to males of the Long-Evans strain. The normal growth curve of the Long-Evans strain overlies that of the Wistar strain and, following the ad-

ministration of anterior hypophyseal extracts, the size of the Long-Evans rats increased until after 180 days of age the growth curve overlies that of the Yale strain.

Since the average adult weight of the Yale strain is 50% heavier than that of the Wistar on the same diet one seems justified in inferring that the growth promoting hormones are operating at a higher level in the Yale strain.

Body fat. The relation of the anterior pituitary to the deposition of body fat is at present a controversial question but an accumulation of experimental and clinical evidence strongly suggests the existence of some relationship (22). In 1922 Evans and Long (23) reported that following the injection of crude anterior hypophyseal extracts into rats, "Increase in weight results to a great extent from a storage of fat, but is not solely due to this, the skeleton being invariably somewhat larger and heavier, . . . " Subsequently, in a carefully controlled series of experiments, Lee and Schaffer (24) demonstrated an increased growth in rats administered anterior pituitary extracts with a decrease in the percentage of body fat. However, these authors placed some restrictions on the food consumption of their animals and used an experimental period, 1/5 to 1/3 as long as those used by Evans and Long, hence, their results do not challenge, necessarily, the accuracy of the former observations concerning the deposition of fat. The higher fat content observed by Evans and Long possibly was influenced by a decreased activity of the gonads resulting from the 200 to 400-day administration of the anterior pituitary. If the apparent contradiction results from the difference in the duration of the injections, the data of the authors comparing the body fat of the two strains provide further evidence of a hyperactivity of the anterior pituitary in the Yale strain. In table 3 the percentage of body fat of the two strains has been compared in 4 age ranges with the result that below 200 days the percentages agree reasonably well but above this age the Yale lead is definite, amounting to 5.8% in the age range 214 to 245 days and

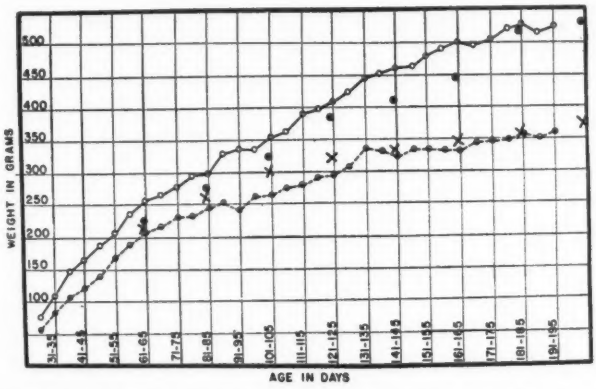

Fig. 2. GROWTH OF MALES, 1938. o——o YALE STRAIN ●----● WISTAR STRAIN (× LONG-EVANS STRAIN ● LONG-EVANS STRAIN ADMINISTERED ANTERIOR PITUITARY EVANS AND SIMPSON '31).

10.0% in the range 294 to 519 days. The Wistar strain showed a remarkably constant percentage of body fat throughout the entire age series studied.

In selecting rats for the fat determinations the individuals were not only paired according to age but in general were required to conform closely to the average growth curves for the strains. Considering the animals under 8 months of age, table 3 shows that the Wistar rats deviate from the mean growth curve by an average of +1.1% while the Yale rats deviate from the mean by an average of −1.4%. Although in the age range 8 to 17 months the deviation from the mean growth curve is greater than in the younger group (Yale, +2 to +26% and Wistar, +7 to +17%) the percentage of body fat in the Yale rat which deviated +2% was the same as that in the Yale rat which deviated +26%. Also the percentage of fat in the Yale rats 8 to 17 months of age is not greatly different from that of the same strain, age 219 to 224 days, each of which is on or a little below the average weight curve. These observations indicate that the difference between the percentage of body fat in the oldest group of the two strains is not dependent upon the slightly greater deviation from the mean growth curve.

TABLE 3. A COMPARISON OF THE BODY FAT OF THE WISTAR AND YALE STRAINS

Wistar males						Yale males					
Rat no.	Age	Body wt.	Deviation from mean growth curve of colony	Nutritive ratio[1] $\frac{W^{1/3}}{L}$	Fat content of body[2] (saponification)	Rat no.	Age	Body wt.	Deviation from mean growth curve of colony	Nutritive ratio[1] $\frac{W^{1/3}}{L}$	Fat content of body[2] (saponification)
	days	*gm.*	%		%		*days*	*gm.*	%		%
162	71	216	+2.4	0.279	9.3	342	71	270	−0.7	0.281	8.6
163	71	226	+7.1	0.282	8.2	341	71	301	+10.7	0.279	7.3
148	112	319	+12.2	0.291	7.5	334	111	391	+1.0	0.283	11.1
157	114	318	+10.4	0.289	7.4	333	111	384	−0.8	0.279	8.7
154	117	314	+7.9	0.290	5.6	337	130	443	+2.3	0.281	12.3
Group av.	97	279	+10.0	0.286	7.6		99	358	+2.5	0.281	9.6
140	146	323	−1.9	0.280	9.4	330	137	450	+0.5	0.285	8.7
123	158	344	+1.2	0.290	12.5	336	160	572	+17.7	0.283	16.0
142	160	321	−5.6	0.279	5.6	319	166	374	−23.6	0.290	9.3
139	162	317	−8.5	0.284	8.1	318	166	451	−8.7	0.284	10.8
Group av.	157	326	−3.5	0.283	8.9		157	462	−3.5	0.286	11.2
112	214	381	+3.5	0.287	6.4	297	219	526	−3.1	0.305	15.1
106	224	375	−0.3	0.285	14.6	299	218	491	−9.6	0.296	15.4
114	226	333	−12.1	0.289	5.2	291	220	521	−4.6	0.284	18.8
108	245	394	−1.5	0.283	12.3	279	224	546	0.0	0.292	12.2
Group av.	227	371	−2.6	0.286	9.6		220	521	−4.3	0.294	15.4
95	393	473	+10.36	0.289	6.0	273	294	799	+26.4	0.307	17.1
92	408	456	+6.79	0.290	8.4	250	373	624	+2.5	0.301	16.3
94	443	500	+17.64	0.284	11.5	203	519	743	+17.9	0.302	22.5
Group av.	415	476	+11.6	0.287	8.6		395	722	+15.6	0.303	18.6
Series av.[3]					8.63						13.14
P.E.m.					±0.47						±0.74

[1] W = weight in gm. L = nose to anus length in cm.
[2] Calculated from the body weight less the weight of the gastrointestinal contents.
[3] The chance that the deviation of the means is due to sampling is 7 in 10,000.

Using the nutritive ratio $\frac{\text{(Weight in grams}^{1/3}\text{)}}{\text{(Nose to anus length in cm.)}}$, employed by Lee (25) to reflect disproportions between weight and length in the rat, it is significant that the difference between the strains parallels the difference between the fat content (table 3). Thus, the values are identical for rats below 130 days of age, averaging 0.283 in the Wistar and 0.281 in the Yale, and for animals in the age range 137 to 166 days the ratios remain essentially the same; 0.283 for the Wistars and 0.286 for the Yales. After 200 days of age the ratios for the Wistar strain change little while those of the Yales increase and in the oldest group the Wistar ratio is 0.287 while the Yale ratio is 0.303. The difference in the oldest group indicates that Yale rats have 20% more weight per cm. of body length. Our Wistar ratios agree well with those recorded by Lee and coworkers (24, 25).

SUMMARY

Rats of the Yale and Wistar strains grown on the same diet and under the same conditions for three generations have been compared. The Yale rats exhibit: a higher fasting blood sugar; a lower glucose tolerance; a greater hyperglycemic response to epinephrine; an initial resistance to insulin; a larger volume of urine per unit of body weight; a more rapid growth and larger size; a greater incidence of sterility and a higher percentage of body fat.

The authors suggest that a hyperfunction of the anterior pituitary in the Yale strain offers a common denominator for the observed differences.

The authors wish to acknowledge their appreciation of the technical assistance of Miss Margaret Jackson and Miss Dorothy Moesta.

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BEHAVIOR AND ANATOMICAL CHANGES PRODUCED IN RATS BY PANCREATECTOMY

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IN THE following experiments a study was made of the effects produced by pancreatectomy on spontaneous activity, food and water intake, vaginal smears and on the endocrine glands of female rats.

METHODS

Spontaneous activity was measured by means of cages described in detail elsewhere (1). Each cage consisted of a revolving drum with a cyclometer, a living compartment with a graduated inverted water bottle and a food cup.

The rats received the standard McCollum diet made according to the following formula.

Graham flour	725 gm.	Butter	50 gm.
Skim milk powder	100 gm.	Sodium chloride	10 gm.
Casein	100 gm.	Calcium carbonate	15 gm.

In this diet carbohydrate amounted to 59.2%; protein, 26.7% and fat, 14.1%.

Records of activity, food and water intake, and vaginal smears were made daily. The animals were weighed weekly.

The rats were placed in the cages at an average age of 47 days, depancreatized at an average age of 65 days, and autopsied 40 days later. At autopsy the ovaries, spleen, adrenals, pituitary, thyroid and thymus were weighed and preserved for histological study. The abdominal cavity was searched for pancreatic tissue with 3 × binoculars.

The operative procedure varied only slightly from that originally used by Shapiro and Pincus (2). Through an incision almost 4 cm. long, slightly to the left of the midline, the tail of the pancreas and the spleen were exposed and the pancreatic tissue was teased away with the fingers. After replacing the spleen, the duodenum and transverse portion of the large intestine were brought into view by means of light traction on the pancreas. Careful dissection between the pancreas and the large intestine resulted in the complete separation of these two organs. The tail and most of the body of the pancreas were rubbed between the finger tips and thus gradually removed. The remaining head and that portion of the body of the pancreas which lies close to the portal system of veins were aspirated by use of a smooth tipped small glass cannula and suction pressure from a water pump. Observation of the entire field of operation with 3 × binoculars and further aspiration resulted in removal of small bits of the gland which had escaped the unaided eye. The proper manipulation of the

[1] Henry Strong Denison Scholar for 1938–1939.

cannula resulted in total extirpation without damage to the large blood vessels or the bile duct. The muscle and skin incisions were closed separately with interrupted black silk sutures.

RESULTS

Survival. Eighteen of the 28 rats died during the 40-day postoperative observation interval; the remaining 10 were alive at the end of this period

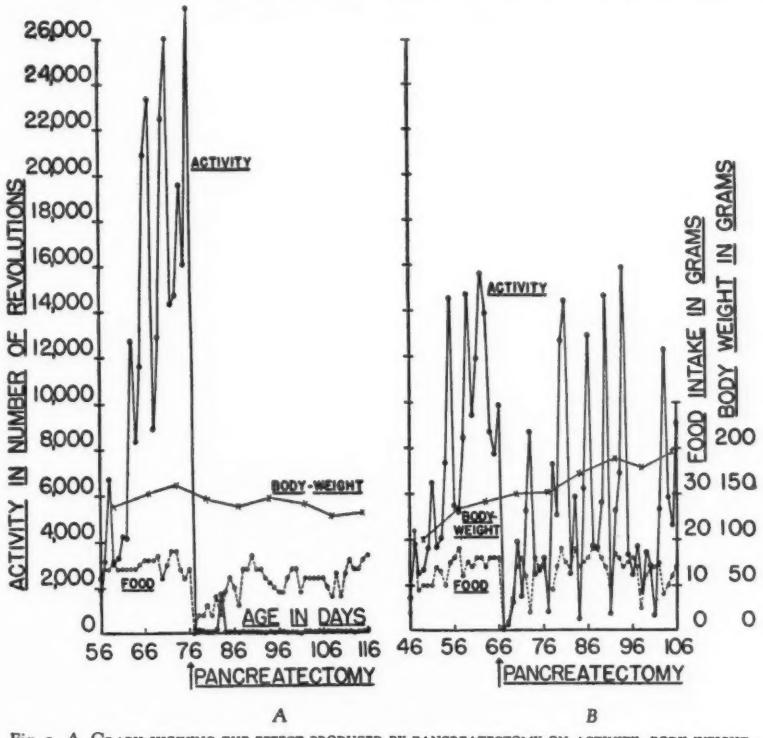


Fig. 1. A. Graph showing the effect produced by pancreatectomy on activity, body weight and food intake in a Group I rat. B. Same for a Group II rat.

and gave all indications of living for a considerably longer time. This report deals only with these 10 animals.

Spontaneous activity. Pancreatectomy greatly reduced the activity of the surviving rats. Six became almost totally inactive (Group I), and 4 showed only a very small amount of activity (Group II). Figure 1*A* gives the activity record of a Group I animal which was placed in the activity cage at 56 days of age and depancreatized 20 days later. During these 20 days the activity increased from 2,000 revolutions per day to a level near 17,000 revolutions. This represents a normal increase in activity for young animals when first placed in the activity cages. Normally the activity would have increased even more during the next 40 days. Pancreatectomy, however, caused the

activity to drop at once to a level of total inactivity at which it remained, with the exception of one day, throughout the following 40 days. Figure 1*B* gives the record of one of the rats of Group II which was placed in the cage at 46 days of age and depancreatized 20 days later. During the 20-day period activity increased from 2,000 revolutions per day to a level near 12,000; and had the animal not been operated upon, the activity would have reached a much higher level during the next 40 days. In this animal pancreatectomy caused the activity to decrease to a lower level, but only for a few days.

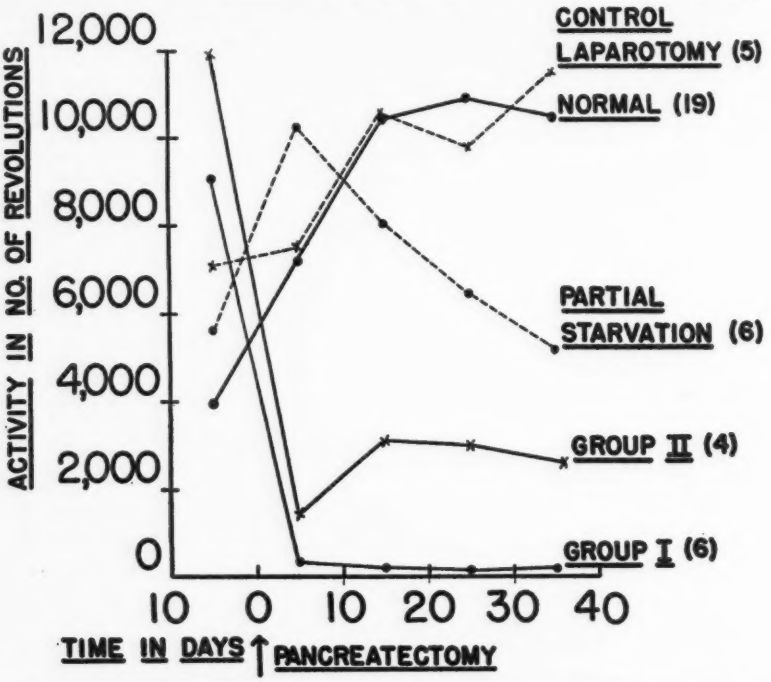


Fig. 2. Daily activity curves in 10-day averages for 19 normal controls, 5 laparotomy controls, 6 partially starved controls, the 6 Group I rats, and the 4 Group II rats.

Thereafter the activity increased again, but showed much wider fluctuations than were present preoperatively, giving a considerably lower average.

The activity curves in 10-day averages for these two experimental groups and for a group of 19 normal unoperated controls of the same age and sex are shown in figure 2.

Table 1 gives the records of the individual animals of each of the two experimental groups. The average daily activity of the 6 rats of Group I decreased from 9,092 revolutions for the 10-day preoperative period to 110 for the 30 to 40-day postoperative period, or a 98.8% decrease. The average daily activity of the 4 rats of Group II decreased from 11,929 revolutions for the 10-day preoperative period to 2,613 for the 30 to 40-day postoperative period, or a 71.8% decrease. The rats of Group I were considerably less active than

hypophysectomized rats, which under the same conditions and operated on at the same age averaged 968 revolutions per day, or an 82.9% decrease (3).

Body weight. The 6 rats of Group I tended to maintain their preoperative weight, 3 showing a small increase and 3 a slight decrease. The weight curve of the animal of this group shown in figure 1*A* decreased somewhat during the 40-day period. The growth rate for the 4 rats in Group II was nearly normal (fig. 1*B*). Table 1 summarizes the results. The 6 rats of Group I averaged 148 gm. at the time of operation and 140 gm. at death, whereas the 4 rats of Group II increased from an average of 142 gm. to 176 gm. 40 days later.

Food intake. In Group I pancreatectomy reduced the food intake in 4 rats and increased it slightly in 2 rats. It reduced the intake of all 4 rats of Group II. Figure 1*A* shows a typical curve. The food intake of this animal decreased from a level of 13 to 14 gm. per day preoperatively to a level of 4

TABLE 1

Rat	Age at operation	Average daily activity in revolutions			Body weight			Average daily food intake			Average daily water intake		
		10-Day preoperative period	30 to 40 Day postoperative period	Per cent decrease	At operation	At death	Ratio of death wt. / operative wt.	10-Day preoperative period	30 to 40-Day postoperative period	Ratio of postop. int. / preop. int.	10-Day preoperative period	30 to 40 Day postoperative period	Ratio of postop. int. / preop. int.
Group I													
1	61	5386	215	96.0	158	162	1.02	11.3	11.5	1.02	16.9	35.1	2.08
2	65	7086	13	99.9	128	132	1.03	12.3	8.5	0.69	19.1	22.5	1.18
3	76	17916	36	100.0	168	140	0.83	15.4	12.9	0.83	24.0	43.5	1.81
4	67	13492	209	98.4	137	118	0.86	11.7	7.5	0.64	22.2	15.6	0.71
5	61	3503	92	97.4	157	167	1.06	10.2	12.5	1.23	26.5	29.5	1.11
6	66	7171	95	98.7	140	121	0.86	9.5	7.0	0.74	21.3	31.0	1.45
Average	66	9092	110	98.8	148	140	0.95	11.7	10.0	0.85	21.6	29.5	1.39
Group II													
1	66	10152	4920	51.5	145	195	1.34	15.3	11.7	0.76	23.5	22.0	0.94
2	66	3342	1382	58.6	144	192	1.33	12.7	11.8	0.93	29.3	35.8	1.22
3	71	18405	3648	80.2	145	165	1.14	13.5	11.2	0.83	30.8	22.4	0.73
4	53	15817	500	96.8	135	150	1.11	12.3	9.9	0.81	17.7	19.5	1.10
Average	64	11929	2613	71.8	142	176	1.23	13.5	11.2	0.83	25.3	24.9	1.00

gm. during the first 10-day postoperative period. After that it increased to a level of 12 gm. at which it was maintained fairly constantly. In figure 1*B* the food intake of one of the Group II rats, like the activity, decreased slightly and showed unusually large daily fluctuations.

Table 1 summarizes these results. The daily food intake of Group I averaged 11.7 gm. for the 10-day preoperative period and 10.0 for the 30 to 40-day postoperative period. In the Group II rats the daily food intake decreased from 13.5 gm. to 11.2 gm. during the same time.

Water intake. Pancreatectomy increased the water intake in 5 of the 6 rats in Group I and had only a slight effect on the 4 rats in Group II. Table 1 summarizes the results. The daily intake of the rats of Group I increased from a preoperative average of 21.6 cc. to a level of 29.5 cc. 30 to 40 days later, giving a ratio of 1.39.

Reproductive tract. Pancreatectomy had a marked effect on the reproductive tract as reflected in the vaginal smears and activity of the Group I animals. The 4 to 5-day cycles of activity and cornified cells disappeared at

once and did not return during the 40-day postoperative observation period. One animal of Group II showed fairly regular 4-day activity and vaginal smear cycles throughout the 40-day period. Another animal showed the 4-day cycles only during the last 20 days; the third animal showed irregular cycles of prolonged periods of cornified smears during the last 20 days; the last animal showed only diestrous smears.

Autopsy findings. (*a*) *Pancreatic tissue.* In the Group I animals there was not sufficient pancreatic tissue present to be recognized as such on gross examination. The Group II animals had one or two small masses which could be recognized as pancreatic tissue, however, these masses were so completely surrounded by fat and connective tissue that they could not be cleared for accurate weighing. Histological study of the tissue which had formed in the location normally occupied by the pancreas showed in the Group I animals only fat, lymph nodes and fibrous tissue. One exception to this occurred in *rat 6* in which small mass of pancreatic tissue of normal appearance was found. However, in the Group II animals a number of the small masses contained both acinar and islet tissue.

TABLE 2. AVERAGE GLANDULAR WEIGHTS

Group	No. animals	Adrenals (Rt.+Lt.)	Thymus	Thyroid	Pituitary	Ovaries (Rt.+Lt.)	Uterus	Ave. age at death
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*days*
Normals	10	46.8	206.8	15.0	11.4	63.1	388.7	101
Group I	6	28.2	97.6	10.0	7.7	37.0	113.0	108
Group II	4	37.0	175.0	14.8	10.8	70.3	345.8	114

(*b*) *Endocrine glands.* The endocrine glands of the 6 rats of Group I weighed less than those of the normals. Table 2 summarizes the results. It gives the average weights of the endocrine glands of the 10 experimental animals and of 10 normal animals killed at approximately the same age. The adrenals of Group I weighed an average of 28.2 mg., as compared to 46.8 mg. for the normals. The weights of the thymus, thyroid, pituitary, uterus and ovaries fell far below the normal averages and were similar to those present after hypophysectomy. In the Group II rats only the adrenals fell far below the normal average.

(*c*) *Blood and urine sugar.* Sugar determinations were made by the method described by Folin and Malmros (4) on blood from the tip of the tail after a 15-hour fast. In as much as this study concerned activity, frequent determinations could not be made. The level of control animals averaged 95 mg.%, which is within the normal range of the rats of other colonies (102.1 mg.%) (2) determined by similar methods, but above that found by Cole and Harned (5) on the Wistar (66 mg.%) and the Yale (85 mg.%) strains of rats by the method of Shaffer, Hartmann and Somogyi, which is known to give lower values than the Folin method. The Group I animals showed considerably elevated blood sugar levels, averaging 178 mg.%, but varying over a fairly wide range. Group II animals averaged 144 mg.%, also with variations.

During the same periods of fasting the urine of control animals had a negative reaction with the qualitative Benedict's reagent, while the urine of the operated animals usually had a 3 plus reaction.

Possible Mechanisms Involved in the Production of Inactivity

The results of these experiments have thus far demonstrated that pancreatectomy made 6 rats almost totally inactive. In addition, it retarded growth, reduced food intake, produced a diestrous condition of the vaginal epithelium, stopped development of the endocrine glands and affected the carbohydrate metabolism.

The question arises now as to the mechanism involved in the production of the inactivity. Several possible answers suggest themselves. First, it is conceivable that the skin incision, the opening through the muscles into the abdominal cavity, the handling of the intestines and the long exposure of the abdominal contents to the air might have produced a shock and, indirectly, a great reduction in activity. Second, the inactivity might be due to the reduced food intake brought about by the pancreatectomy. Third, since from previous experiments we know that the endocrine glands, the gonads, adrenals, thyroid and the pituitary play an important part in the control of activity, inactivity might have resulted from a reduced secretion from these glands. And fourth, the inactivity might have had its origin in the changes in carbohydrate metabolism, the inability of the pancreatectomized rats to use carbohydrate.

The following experiments were undertaken in an attempt to test these different hypotheses.

(*a*) *Effects produced on activity by control laparotomy operations.* Under standard conditions we subjected 5 female rats to exactly the same operative procedure as that involved in pancreatectomy, except that we did not remove the pancreas. We operated when the rats averaged 58 days and killed them 40 days later. In this operation we made the skin incision, the opening through the muscles into the abdominal cavity, exposed the pancreas, and freed it from the intestines wherever the circulation and secretory activity of the gland would not be disturbed. The operation required approximately the same length of time (about 20 minutes) as the regular pancreatectomy.

This operation had practically no effect on activity. After a few days of reduced activity caused by surgical trauma, the animals quickly regained normal activity levels (fig. 2). The average daily activity curves from the 19 normal females and 5 females with control laparotomies closely approximate one another. Food and water intake, body weight, vaginal smears and the weights of the endocrine glands also remained unaffected.

Thus, the operative procedure, incidental to the actual removal of the pancreas, did not make the rats inactive.

(*b*) *The rôle played by the reduced food intake.* In order to determine whether the reduced food intake could account for the inactivity of the pancreatectomized rats, we undertook the following experiment. We gave 6 normal 60-day old rats only 8 gm. of the regular food per day, an amount which approximated the average daily intake of the pancreatectomized ani-

mals. We recorded their activity, etc., for 40 days, then autopsied them.

In sharp contrast to the pancreatectomized rats, these animals on the partial starvation diet remained fairly active (fig. 3). For the first 10 days when this rat had access to unlimited amounts of McCollum diet, it ate approximately 13 gm. per day, and its activity averaged 8,000 revolutions per day. On the restricted diet, its activity continued to increase, reaching a level of 16,000 revolutions after 10 to 20 days. From then on activity decreased again, but still maintained a fairly high level at the end of the 40-day period. Figure 2 gives the averages and shows the relationship between the

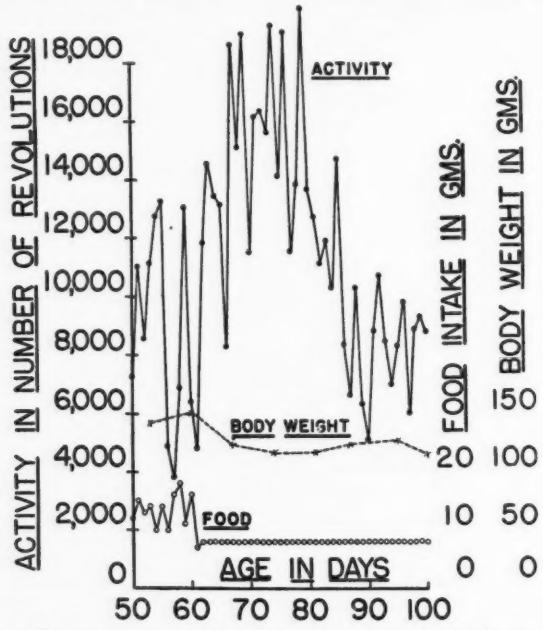


Fig. 3. Graph showing the effect produced on activity of one animal by partial starvation diet (8 gm. per day).

activity of these control rats and the activity of the Group I and II experimental rats.

Four of the 6 partially starved animals showed cornified smears irregularly throughout the 40-day period; 2 animals showed cornified smears irregularly for 8 days and thereafter constant diestrous smears.

Clearly, the reduced food intake did not produce the inactivity of the pancreatectomized animals.

(c) *The rôle played by the endocrine glands.* The results reported above showed that in the Group I animals the endocrine glands weighed far less than those of the normal controls. Since, however, the body weights averaged considerably less than those of the controls, we could not conclude, without a knowledge of the weight per kg. of body weight, that the glands actually had atrophied. Table 3 contains the weights of the various glands per kg. of body weight of the Group I and II rats, of the 10 normal female rats, and of

the 6 partially starved rats, all of them killed at approximately the same age. A comparison of the percentage of body weight values for the glands of the normal and of the Group I rats shows that all had atrophied—the thyroid and the pituitary only very slightly, the adrenals, thymus and ovaries considerably. Thus, when calculated as percentage of body weight, the adrenals of normals gave a value of 0.029 and of Group I rats, only 0.020; the thymus of normal rats gave a value of 0.124 and of the Group I rats, 0.069; the ovaries of normals, 0.083 and of Group I rats, 0.026.

On the basis of these records it would appear that the reduced activity of the pancreatectomized rats might have been due to this marked atrophy of the endocrine glands, particularly the adrenals and ovaries. We found, however, that the 6 partially starved rats which were quite active showed almost the same degree of atrophy of all of the glands, with the exception of the ovaries. The ovaries of the Group I rats were definitely smaller, 0.026

TABLE 3. AVERAGE GLANDULAR WEIGHTS EXPRESSED AS PERCENTAGE OF BODY WEIGHT

Group	No. rats	Adrenals (Rt. +Lt.)	Thymus	Thyroid	Pituitary	Ovaries (Rt. +Lt.)	Uterus	Pancreas	Ave. age in days at death
Normals	10	0.029	0.124	0.009	0.007	0.083	0.233	0.393	101
Group I	6	0.020	0.069	0.007	0.005	0.026	0.081		108
Group II	4	0.019	0.099	0.008	0.006	0.040	0.197		114
Partial Starvation	6	0.021	0.106	0.008	0.006	0.035	0.114	0.469	100

as compared to 0.038. Since we know that even total removal of the ovaries would not have resulted in such a great decrease in activity, it would appear that still some other factor must play a more important part (6).

A further study of the Group I rats and of the partial starvation controls disclosed the fact that the presence of the pancreas in the partially starved group and its absence in the experimental group constituted the chief difference between these two groups. Table 3 shows that in the partially starved group the pancreas not only failed to show an atrophy like the other glands, but on the contrary, showed a definite hypertrophy. The pancreas weight averaged 0.469% as compared to 0.393% in normals.

It would appear, then, that the presence of the pancreatic secretion in the partially starved rats accounted for the high level of activity as compared to the Group I rats.

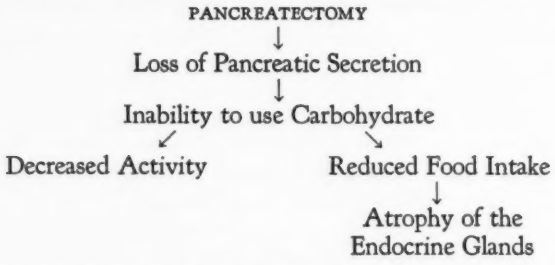
(d) *The rôle played by the disturbance in carbohydrate metabolism.* From the foregoing results it appears that the pancreatectomized rats became inactive, not because of changes in the endocrine glands or of reduced food intake, but directly because of the loss of the pancreas.

From our knowledge of the importance of carbohydrate in the production of activity (7) and of the disturbance in carbohydrate metabolism produced in depancreatized animals, it appears that the Group I rats might have become inactive because of the reduced ability to utilize carbohydrate.

The reduced food intake also appears to depend on the disturbance in carbohydrate metabolism; that is, since the rats are unable to use the normal amount of carbohydrate, they reduce their food intake to a lower level. The

reduced food intake, then, in turn may produce the loss of weight and atrophy of the endocrine glands.

The following schema shows how the relationship between these different phenomena may be formulated on the basis of the results reported above:



The acidosis which results from the inability to use carbohydrate may also help to reduce activity, as may also the loss of external pancreatic secretion.

SUMMARY

At an average age of 65 days 28 rats were pancreatectomized. Eighteen died and 10 survived during the 40-day observation period.

Six of the 10 surviving rats became almost totally inactive. Their average daily activity decreased 98.8%. The other 4 became only moderately inactive, showing a decrease of 71.8%.

The 6 totally inactive rats stopped growing but tended to maintain their operative weight; they ate less food, drank more water, showed diestrous vaginal smears, and had small endocrine glands. The 4 moderately inactive rats deviated only slightly from the normal in body weight, water intake and vaginal smears.

At autopsy, performed 40 days after pancreatectomy, 5 of the 6 inactive rats showed no pancreatic tissue; the other 4 rats, minute fragments.

Control laparotomy experiments showed that the operative manipulation did not make the rats inactive.

Control experiments were made with 6 rats kept on a limited diet of 8 gm. per day (the average daily intake of the pancreatectomized rats). These rats showed a high degree of activity, thus suggesting that the pancreatectomized rats did not become inactive because of their reduced food intake.

Atrophy of the endocrine glands, with the possible exception of the ovaries, and reduced food intake were ruled out as causal factors in producing the inactivity.

It was concluded that the depancreatized rats became inactive largely because of their inability to metabolize carbohydrate.

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FURTHER OBSERVATIONS ON THE EFFECT OF THYROGLOBULIN AND THYROXIN ON THE O_2 CONSUMPTION OF TISSUES IN VITRO[1]

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IN A PREVIOUS paper[2] we reported the effect on liver metabolism of various substances which have a putative relationship to the thyroid hormone, namely; thyroglobulin, thyroxin, diiodothyronin, thyronin and diiodotyrosin. Of these substances, only thyroglobulin had an unequivocal effect in elevating the O_2 consumption of the liver in vitro. We have extended the observations on thyroglobulin and thyroxin to other tissues, with the object of determining whether the peculiarities of response of the individual tissues may throw further light on the nature of the thyroid hormone. The present communication deals with the guinea pig kidney, testis and heart.

METHODS

The O_2 consumption of the tissues was determined in a Barcroft-Warburg differential manometer system. The kidney and heart were sliced free hand, as rapidly as possible after removal from animals which were killed by a blow on the head. Suitable precautions were taken as to thickness of slices. In the case of testis, the tissue was teased apart. The duration of the experiments was one hour. Three different types of medium were used in the respiratory flasks: (*a*) 0.85% NaCl in a Sörensen buffer mixture with M_{180} glucose, the PO_4 being in either M_{150} or M_{75} concentration: (*b*) Ringer's solution in similar concentrations of PO_4 and glucose; (*c*) horse serum, made almost bicarbonate free by being shaken with HCl at a pH of 6.0 to 6.5, then brought to physiological pH with NaOH. This serum contained no fermentable sugar, and had about 50 mg.% reducing substances. In some experiments glucose was added to the serum to a concentration of M_{180}.

The pH was determined with a glass electrode before and after the experiment. The usual starting pH was about 7.4, and with rare exceptions did not go below 7.0 during the experiment. In view of the effects of pH on the O_2 consumption of isolated tissues, experiments where the pH fell to unphysiological levels were not used. This occurred chiefly in the experiments on testis, in which, as in brain, there is a marked tendency toward lowering of the pH in phosphate buffer solutions.

Whenever possible, the effect of thyroglobulin and thyroxin was de-

[1] Aided by a grant from the E. P. Charlton Research Fund. Tufts College Medical School, Boston.
[2] CANZANELLI A., AND D. RAPPORT: *Endocrinology* 21: 779. 1937.

termined on the tissue from the same animal (in the tables, experiments bearing the same date are of such a nature), for the evidence is clear that tissues from different animals respond differently to changes in the medium. The initial level of oxygen consumption, which, as we shall develop later, is a factor in this response, also varies widely in different animals, the reasons remaining obscure. We may say, incidentally, that in the experi-

TABLE 1. KIDNEY

Date	Medium	Amt. test substance added	QO_2		
			Control	Test	Change %
Thyroglobulin					
Oct. 31, '38	NaCl/gl/$M_{150}PO_4$	8.5 mg.	17.3±0.4	18.1	+ 5
Nov. 1, '38	NaCl/gl/$M_{150}PO_4$	7.0 mg.	11.5	16.2±0.2	+41
Dec. 13, '38	NaCl/gl/$M_{75}PO_4$	5.4 mg.	11.7±0.4	17.3±0.4	+48
Jan. 24, '39	NaCl/gl/$M_{75}PO_4$	9.5 mg.	19.5±0.4	19.1±0.4	− 2
Feb. 8, '39	NaCl/gl/$M_{75}PO_4$	10.0 mg.[1]	13.1±0.5	15.2±1.2	+16
Nov. 7, '38	Ringers/gl/$M_{150}PO_4$	6.6 mg.	14.5±0.5	16.5±0.5	+14
Nov. 21, '38	Ringers/gl/$M_{75}PO_4$	5.8 mg.	17.7±0.3	18.7±1.1	+ 6
Dec. 1, '38	Ringers/gl/$M_{75}PO_4$	10.0 mg.[1]	20.0±1.0	17.6±0.1	−12
Dec. 9, '38	Ringers/gl/$M_{75}PO_4$	6.2 mg.	18.3±0.1	18.0±0.3	− 2
Dec. 14, '38	Ringers/gl/$M_{75}PO_4$	6.6 mg.	12.1±0.5	17.9±0.2	+48
Feb. 8, '39	Ringers/gl/$M_{75}PO_4$	10.0 mg.[1]	11.6±0.3	13.5±0.6	+16
Nov. 10, '38	Serum	14.4 mg.	20.5±0.6	22.9±0.8	+12
Nov. 11, '38	Serum	10.0 mg.[1]	18.2±0.4	22.2±0.1	+22
Nov. 15, '38	Serum+M_{180}glucose	10.0 mg.[1]	17.8±0.9	20.9±0.3	+17
Nov. 17, '38	Serum	10.0 mg.[1]	18.4±0.5	21.6±1.3	+17
Dec. 16, '38	Serum	8.4 mg.	21.1±0.7	24.1±1.1	+14
Thyroxin					
Oct. 31, '38	NaCl/gl/$M_{150}PO_4$	123γ	17.3±0.4	15.5±0.4	−10
Nov. 1, '38	NaCl/gl/$M_{150}PO_4$	68γ	11.5	12.4±0.3	+ 8
Dec. 13, '38	NaCl/gl/$M_{75}PO_4$	121γ	11.7±0.4	11.3±1.0	− 4
Jan. 24, '39	NaCl/gl/$M_{75}PO_4$	44γ	19.5±0.4	14.5±1.8	−26
Nov. 7, '38	Ringers/gl/$M_{150}PO_4$	23γ	14.5±0.5	13.5±0.3	− 7
Nov. 21, '38	Ringers/gl/$M_{75}PO_4$	42γ	17.7±0.3	13.6±1.2	−24
Dec. 1, '38	Ringers/gl/$M_{75}PO_4$	?[1]	20.0±1.0	15.3±0.6	−24
Dec. 9, '38	Ringers/gl/$M_{75}PO_4$	81γ	18.3±0.1	14.2±1.2	−22
Dec. 14, '38	Ringers/gl/$M_{75}PO_4$	87γ	12.1±0.5	10.8±0.3	−11
Nov. 10, '38	Serum	163γ	20.5±0.6	17.0±0.4	−12
Nov. 11, '38	Serum	193γ	18.2±0.4	22.3	+23
Nov. 15, '38	Serum+M_{180}glucose	267γ	17.8±0.9	19.2±1.2	+ 8
Nov. 17, '38	Serum	230γ	18.4±0.5	19.8±0.1	+ 8
Dec. 16, '38	Serum	196γ	21.1±0.7	22.0±0.3	+ 4

[1] I_2 determinations not made. The figure represents the approximate amount added.

ments reported below this variation was not a function of the previous effect of changes in environmental temperature to which the animals were subjected, for we were at pains to keep this constant within 70 to 75°F. for a period of from 48 hours to several weeks before the animals were killed.

Thyroglobulin and thyroxin were used in iodine concentrations of a similar order of magnitude. About 200γ of thyroxin was usually added to each flask, the amount actually in solution at physiological pH as shown by I_2 determinations, varying with the medium, being greatest in serum, as may

TABLE 2. TESTIS

Date	Medium	Amt. test substance added	QO_2		
			Control	Test	Change %
Thyroglobulin					
Nov. 2, '38	NaCl/gl/$M_{150}PO_4$	6.7 mg.	9.2±0.2	9.1±0.2	− 1
Dec. 20, '38	NaCl/gl/$M_{75}PO_4$	5.4 mg.	6.8±0.4	8.5±0.5	+25
Feb. 3, '39	NaCl/gl/$M_{75}PO_4$	20.0 mg.[1]	9.6±0.5	8.4±0	−12
Nov. 9, '38	Ringers/gl/$M_{75}PO_4$	4.5 mg.	8.6±0.3	9.3±0.1	+ 8
Nov. 18, '38	Ringers/gl/$M_{75}PO_4$	5.6 mg.	6.9±0.6	8.0±0.1	+16
Nov. 16, '38	Serum+M_{180}glucose	22.6 mg.	9.0±0	9.5±0.2	+ 6
Feb. 3, '39	Serum	18.5 mg.	9.3±0.1	9.9	+ 6
Thyroxin					
Nov. 2, '38	NaCl/gl/$M_{150}PO_4$	75γ	9.2±0.2	7.9±0.2	−14
Dec. 20, '38	NaCl/gl/$M_{75}PO_4$	77γ	6.8±0.4	5.7±0.1	−16
Feb. 3, '39	NaCl/gl/$M_{75}PO_4$	77γ	9.6±0.5	7.0	−27
Nov. 3, '38	Ringers/gl/$M_{150}PO_4$	56γ	9.3±0.7	6.6±0	−29
Nov. 9, '38	Ringers/gl/$M_{75}PO_4$	51γ	8.6±0.3	7.9±0	− 8
Nov. 18, '38	Ringers/gl/$M_{75}PO_4$	44γ	6.9±0.6	7.2±0.3	+ 4
Nov. 16, '38	Serum+M_{180}glucose	235γ	9.0±0	9.0±0.3	0
Feb. 3, '39	Serum	381γ	9.3±0.1	9.7±0.4	+4

[1] I_2 determinations not made. The figure represents the approximate amount added.

be seen in the tables. Within the limits used, the effect did not appear to be a function of the concentration of either of these substances.

RESULTS

Kidney. Only one kidney was used for each experiment, the pooled slices containing both cortical and medullary tissue. The effects of thyroglobulin are summarized in table 1. In the NaCl/glucose/PO_4 mixture, the effect of thyroglobulin depended on the initial level of O_2 consumption by the tissue; varying from no effect at all when the control QO_2 was 19.5 to increases of 41 and 48% when the control QO_2's were 11.5 and 11.7 respectively. The phosphate concentration did not appear to affect these results. In Ringer's/glucose/PO_4, thyroglobulin again had no influence on the QO_2 when the level of metabolism of the tissue was at a QO_2 of 18 or above; but appeared at lower levels, although in one case, the increase due to thyroglobulin was only 16% when the control QO_2 was as low as 11.6. In serum the O_2 uptake was generally at a higher level than in either of the salt mixtures, ranging from 18 to 21, a phenomenon we have observed in other experiments on kidney. However, even at these high levels of initial metabolism thyroglobulin, in the serum medium, was still able consistently to elevate the metabolism further, the average increase being about 18%.

Thyroxin, in both of the salt media, had no tendency to elevate the O_2 consumption, and at the higher levels of initial metabolism tended to depress it, as may be seen in table 1. In serum there was in 4 out of 5 experiments no evidence of a stimulating effect on the O_2 uptake. In one experiment, however, the QO_2 was distinctly elevated by thyroxin.

TABLE 3. HEART

Date	Medium	Amt. test substance added	QO_2 Control	QO_2 Test	QO_2 Change %
Thyroglobulin					
Jan. 4, '39	NaCl/gl/$M_{150}PO_4$	8.8 mg.	5.1	6.9±0.7	+ 35
Jan. 9, '39	NaCl/gl/$M_{150}PO_4$	10.0 mg.[1]	3.2	5.4±0	+ 69
Jan. 10, '39	NaCl/gl/$M_{150}PO_4$	9.0 mg.	4.5	5.9±0.8	+ 31
Jan. 11, '39	NaCl/gl/$M_{75}PO_4$	10.0 mg.[1]	6.0±0.2	9.6±0.4	+ 60
Jan. 12, '39	NaCl/gl/$M_{75}PO_4$	9.0 mg.	3.8±0.2	7.8±0.9	+105
Nov. 22, '38	Serum	10.0 mg.[1]	14.7±1.1	14.7±1.0	0
Nov. 23, '38	Serum	11.8 mg.	7.8±0	10.2±0.3	+ 31
Dec. 6, '38	Serum	10.2 mg.	8.8±0.4	10.1±1.1	+ 15
Dec. 21, '38	Serum	9.5 mg.	5.5±0.1	6.0±0.4	+ 9
Dec. 28, '38	Serum	9.2 mg.	9.6±0.2	11.3±0.2	+ 18
Jan. 19, '39	Serum	10.6 mg.	7.2	9.9±1.3	+ 38
Jan. 25, '39	Serum	20.3 mg.	7.9±0.3	11.4	+ 44
Thyroxin					
Jan. 4, '39	NaCl/gl/$M_{150}PO_4$	32γ	5.1	3.2±0.2	−37
Jan. 9, '39	NaCl/gl/$M_{150}PO_4$	44γ	3.2	3.6±0.2	+13
Jan. 10, '39	NaCl/gl/$M_{150}PO_4$	44γ	4.5	4.9±0.4	+ 9
Jan. 11, '39	NaCl/gl/$M_{75}PO_4$	38γ	6.0±0.2	5.3	−12
Jan. 12, '39	NaCl/gl/$M_{75}PO_4$	41γ	3.8±0.2	3.8±0.3	0
Nov. 22, '38	Serum	226γ	14.7±1.1	15.5±0.2	+ 5
Nov. 23, '38	Serum	230γ	7.8±0	8.9±0.2	+11
Dec. 6, '38	Serum	166γ	8.8±0.4	9.3±0.8	+ 6
Dec. 21, '38	Serum	189γ	5.5±0.1	5.1±0.1	− 7
Dec. 28, '38	Serum	184γ	9.6±0.2	8.4±0.3	−12
Jan. 19, '39	Serum	221γ	7.2	10.7±0.5	+49
Jan. 25, '39	Serum	390γ	7.9±0.3	7.6±0.1	− 4

[1] I_2 determinations not made. The figure represents the approximate amount added.

Testis. When the initial QO_2 in NaCl was in the neighborhood of 9, thyroglobulin had no effect, (table 2), but at an initial QO_2 of 6.8, it elevated the O_2 consumption by 25%. In Ringer's, at a control QO_2 level of 8.6, thyroglobulin caused an increase of only 8%; at a control level of 6.9, the rise due to thyroglobulin was 16%. The latter, though small, was undoubtedly authentic, as the error in the testis experiments was always a small one. In serum, at QO_2 levels of about 9, thyroglobulin had no effect.

In no case did thyroxin produce an elevation of O_2 consumption by the testis, and in the salt media almost consistently depressed it, particularly at the higher levels of metabolism.

Heart. It was at times difficult to obtain sufficient tissue for observations from a single guinea pig heart, and in some experiments slices from two or even three hearts were pooled. In the NaCl media the addition of thyroglobulin caused a consistent and marked increase in O_2 consumption (table 3). This was present even at the highest levels of metabolism, and the correlation between the height of the control metabolism and the percentage increase due to thyroglobulin was not as definite as in the other tissues. In the serum medium, one experiment showed a control metabolism extraordinarily high for guinea pig heart, the QO_2 being 14.7, and in this case

thyroglobulin had no effect whatever. In the other serum experiments, the O_2 uptake was in all cases (except one doubtful one) increased by thyroglobulin, the increases ranging from 9% to 44%.

Thyroxin, as in the case of the other tissues, did not stimulate the metabolism except in one of the 7 serum experiments. In one experiment in the salt medium, there was apparently a depressing effect of thyroxin at what, for heart in this medium, was a relatively high metabolic rate.

DISCUSSION

The main conclusion to be derived from these experiments is that under certain conditions thyroglobulin acts as definite stimulant to the aerobic oxidations in kidney, testis and heart, just as it did in our earlier observations on liver. The conditions appear to be that the metabolic rate of the tissue before the addition of thyroglobulin shall not be at a very high level. There seems to be a 'ceiling' above which thyroglobulin cannot further elevate metabolism. This 'ceiling,' except in testis, where the control O_2 uptake is very little different in the two types of media, is higher in serum than in the salt buffer mixtures. We have performed several further experiments on guinea pig liver in this connection, and may state that in the salt buffer mixture we obtained results similar to the ones we have previously reported, in which thyroglobulin consistently elevated the metabolism. In serum, however, a 'ceiling' was evident, above which level thyroglobulin had no apparent effect.

Thyroxin, in contrast to thyroglobulin, in no case caused an unequivocal rise in O_2 consumption of any of the tissues in the salt buffer mixture, and at the higher metabolic levels there was a tendency towards a depressive effect in heart and testis. Although, in the serum experiments, there was for the most part no evidence of a stimulating action of thyroxin, nevertheless in one observation on kidney and in another on heart, the authenticity of which we have no reason to doubt, the QO_2 was distinctly elevated by thyroxin. This is not a function of the concentration of thyroxin, which was the same as in the other serum experiments, and we must therefore consider the possibility that under certain conditions the physiological properties of thyroxin may be altered by contact with serum.

SUMMARY

The effect of thyroglobulin and thyroxin upon the in vitro O_2 consumption of guinea pig kidney, testis and heart was investigated. At lower levels of metabolism thyroglobulin stimulated the metabolism of each of these tissues. However, there appeared to be a 'ceiling' above which thyroglobulin could no further elevate the O_2 uptake, and this 'ceiling' was higher, in the case of kidney and heart, in serum than in a salt buffer mixture.

Thyroxin generally had no stimulating effect in either the salt buffer mixture or the serum. In two experiments in serum, however, a stimulating effect appeared, raising the possibility that thyroxin under certain circumstances may be made physiologically active by contact with serum.

THE RELIEF OF MYXEDEMA WITH PROTEINS OF EXTRATHYROIDAL ORIGIN

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RECENT STUDIES of the effects of the pituitary and of iodine administration upon the thyroid gland have focussed our attention upon the composition, storage and release of active hormone by the thyroid. Further progress along these lines awaits better understanding of the chemical machinery and physiological actions which these processes manifest in the building up and breaking down of thyroid protein.

The isolation of thyroxin, by Kendall, led to the conception that the prime function of the gland is the manufacture of this substance in toto, that is a thyronine nucleus, as described by Harington (1), equipped with a complement of 4 iodine atoms to make tetraiodothyronine, which is thyroxin. This substance is then supposedly anchored by incorporation into thyroid protein as described by Harington and Salter (2). The studies to be reported, however, show that the simple incorporation of iodine into nondescript protein leads to thyroidal activity.

When blood serum protein, in ammoniacal solution, is treated with compound solution of iodine according to the procedure of Wormall (3), within a short time there results an iodo-protein. The iodine content, totalling 15 to 20%, is organically bound. The material is then separated by repeated precipitation at its isoelectric point of PH 5.0. The technic is more fully described in a previous paper (4).

EFFECT OF IODOPROTEIN

The iodoprotein was administered to 4 patients with athyreosis. Rapid and complete recovery occurred with proper dosage, as shown in figure 1. The clinical appearance and behavior of these patients became normal. Diuresis occurred and blood cholesterol dropped. The dosage varied from 60 mg. to 420 mg. of protein iodine daily, dissolved in dilute alkali and buffered with sodium bicarbonate. The latter dosage made one patient (C. S.) definitely thyrotoxic. This patient also began to menstruate on the fifth day of therapy after 5 years of amenorrhea. Another patient (*B. G.*), a 5-year-old patient with cachexia strumipriva, previously described (4), made a standard response on half the adult dose. The dosage in terms of iodine is some 400 times as large as that of thyroglobulin or desiccated thyroid.

As a control observation, 2 patients received non-iodinated serum protein

* Aided by a grant from the International Cancer Research Foundation.

in dosage corresponding to the maximum amount of iodoprotein used in figure 1. They showed no effect. This protein came from the same source of serum as the iodoprotein. The maximum amount of iodine originally present in the untreated serum was 0.003 mg. per dose.[1] It is obvious, therefore, that preformed thyroxin cannot account for the effect noted.

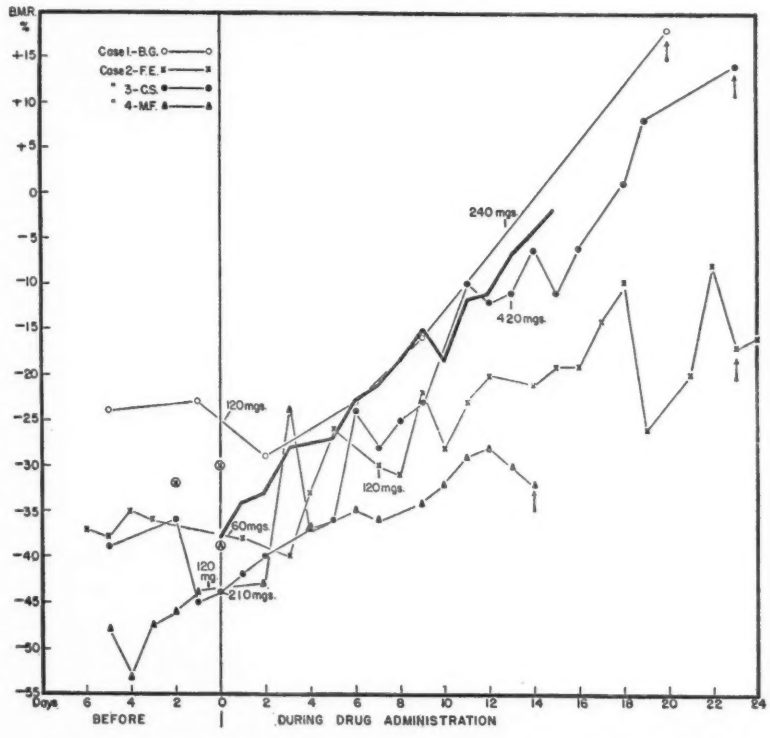

Fig. 1. THE METABOLIC RESPONSE OF 4 PATIENTS WITH MYXEDEMA RECEIVING IODOPROTEIN IN DOSAGE VARYING FROM 60 TO 420 MG. OF IODINE DAILY. The arrows indicate omission of medication. The heavy solid line represents the metabolic response to the daily administration of thyroxin polypeptide containing 0.5 mg. of iodine. The response of *F. E.* and *M. F.*, on dosage of 60 to 120 mg. of iodine, was below standard; the response of *C. S.* to 210 mg. of iodine was standard; and the response of *B. G.*, a child of 5, was standard to 120 mg. of iodine.

CONSTITUENTS OF IODOPROTEIN

Qualitatively, apart from dosage, no clinical or metabolic differences have been observed between (*a*) the above iodinated protein, (*b*) natural thyroglobulin and (*c*) the artificial thyroid colloid which Salter and Lerman (5) previously studied. The discrepancy in dosage suggests that much inert iodine is present in the first of these. Like the last two proteins, the iodinated protein can be fractionated by enzymes or other hydrolytic agents into

[1] For this analysis we are indebted to Mr. H. J. Perkin of the Lahey Clinic.

smaller fragments. When it is treated with pepsin, for example, the iodinated protein is split into two sorts of peptones. One of these, designated in this report as the 'T' peptone, behaves like the corresponding fraction derived

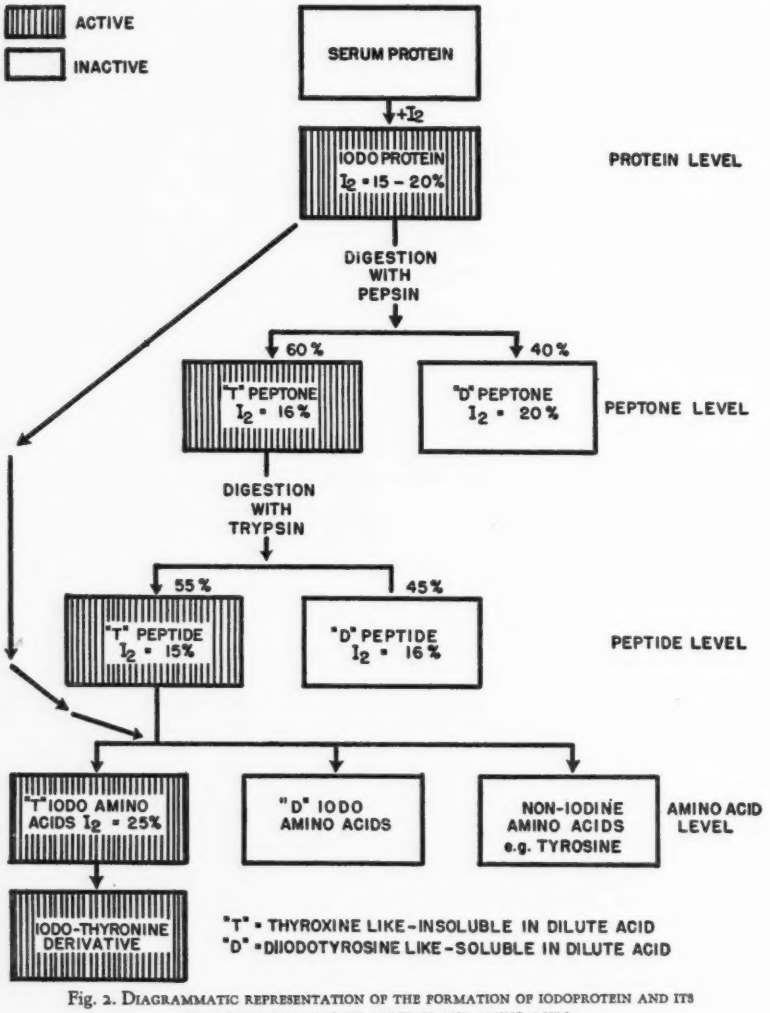

Fig. 2. Diagrammatic representation of the formation of iodoprotein and its hydrolysis to peptones, peptides and amino acids.

from thyroglobulin. It is insoluble in weak acid and is highly potent. The other fraction, designated here as the 'D' peptone, behaves like the diiodotyrosine peptone derived from thyroglobulin and is soluble in weak acid but metabolically inert. This fractionation is illustrated in figure 2.

The T peptone was tested in 3 patients with hypothyroidism. It was also dissolved in dilute alkali and buffered with sodium bicarbonate. Two of these responses are shown in figure 3. One patient (*M. D.*) made the standard response expected from 0.5 mg. of thyroid iodine. She received all of

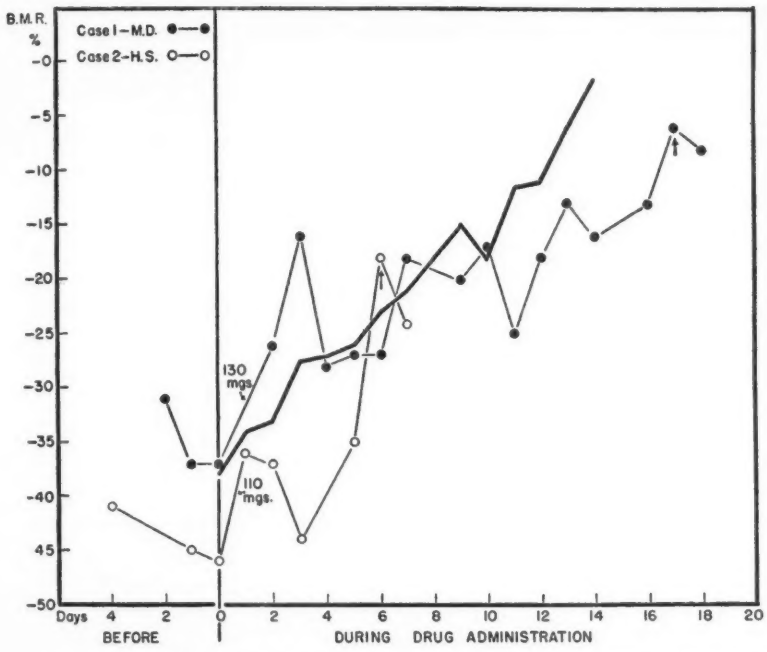

Fig. 3. THE METABOLIC RESPONSE OF 2 PATIENTS TO THE 'T' PEPTONE FRACTION. The patient *M. D.*, with myxedema, made a standard response to 130 mg. of iodine, which represents all the iodine recovered from a standard dose of iodoprotein (210 mg. of iodine) by peptic hydrolysis. The assay on the other patient was interrupted after the 6th day, as explained in the text.

the T iodine (130 mg.) derived quantitatively from a standard dose of original iodinated protein containing 210 mg. of iodine, as indicated in table 1. Thus no activity was lost in the chemical fractionation. The other patient (*H. S.*) shown in figure 3, received the T fraction in a dose of 110 mg. of iodine

TABLE 1. COMPOSITION OF FRACTIONS OBTAINED BY PEPTIC HYDROLYSIS OF IODINATED HORSE SERUM PROTEIN

Patient	Iodine content of single dose of iodoprotein	Iodine recovered as 'T' peptone[1]		Iodine recovered as 'D' peptone[1]		Total recovery[2]
	mg.	mg.	%	mg.	%	%
M. D.	210	130	65	72	35	97
B. G.	210	113	59	80	41	93
H. S.	210	110	59	87	41	90

[1] These figures represent percentage of yield.
[2] These figures represent percentage of starting material recovered.

daily for 6 days only. Her metabolism rose to −18%. To our surprise she became comatose. Immediate study proved that she was suffering from acute adrenal insufficiency. In short, it became apparent, from this and other considerations, that her myxedema was secondary to pituitary deficiency and that active medication had precipitated an Addisonian crisis. Such an effect is cited by Means in his textbook (6) and is characteristic of active thyroid material under these circumstances.

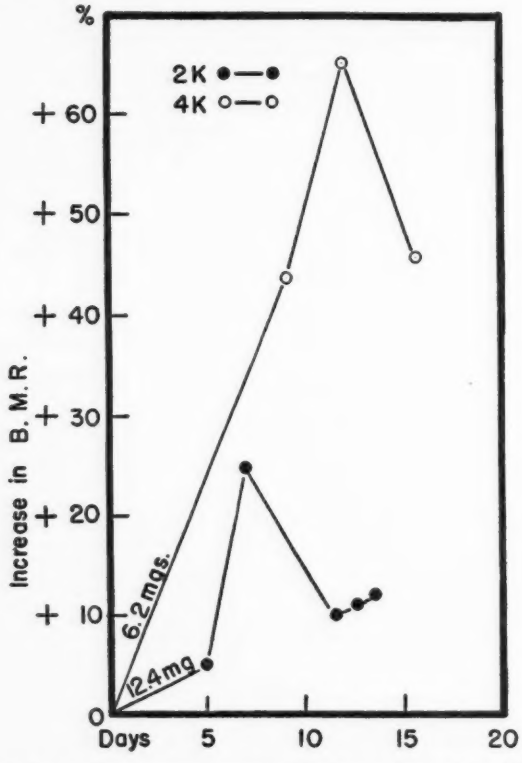

Fig. 4. THE RESPONSE OF 2 MYXEDEMATOUS RABBITS TO 'T' PEPTONE. One, 4K was fed 6.2 mg. of iodine every other day for 7 doses; the other, 2K was fed 12.4 mg. of iodine every other day for 9 doses.

The third patient (*B. G.*), not charted, was maintained at a normal level of metabolism (+7%) by a daily dose of 113 mg. of iodine for 20 days.

The T peptone was also tested in 2 myxedematous rabbits (fig. 4). One animal (4K) responded rapidly to 45% above his myxedematous level; the other (2K), did not respond as well, reaching a level of only 12% above the original state on twice as much iodine. However, the material this animal received had remained in the ice box about two weeks after its administration to the first animal. A fortnight later, this same material failed

to produce any response whatever in a myxedematous patient. Consequently, slow deterioration is suggested.

The D peptone, obtained by the above procedure, was tested in patient *H. S.* and found to be inert.

It is possible to fractionate the T peptone further, as indicated in figure 2, by means of trypsin or alkali. Trypsin separates the T peptone into a T peptide containing 15% iodine and a D peptide containing 16% iodine. It would be expected that the T peptide would have a high activity whereas

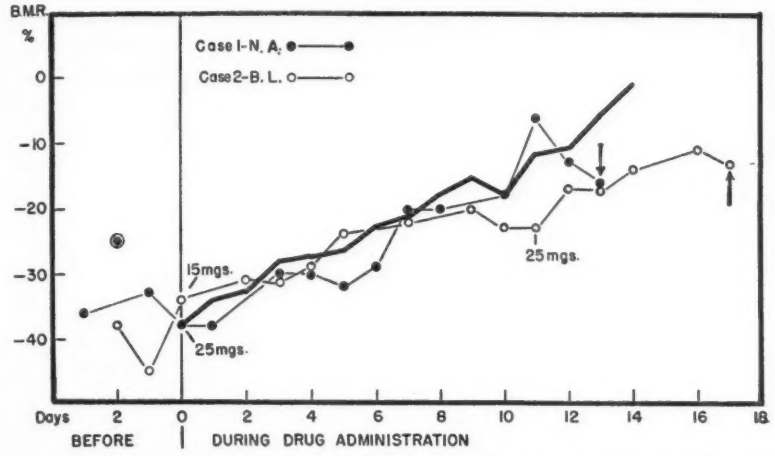

Fig. 5. THE METABOLIC RESPONSE OF 2 PATIENTS WITH MYXEDEMA TO CRYSTALLINE 3:5 DIIODOTHYRONINE. One patient, *N. A.*, received a daily oral dose of material containing 25 mg. of iodine; the other, *B. L.*, received a daily oral dose of material containing 15 mg. of iodine which was increased to 25 mg. of iodine on the 11th day.

the D peptide would be ineffective. Indeed, the standard curve of reference used in the charts of this paper is based on the response to a similar peptide which was derived from thyroglobulin. One patient (*B. G.*) attained a metabolism of +20% on daily administration of 72 mg. of iodine of T peptide for 2 weeks. This amount of peptide represents the complete recovery from 210 mg. of iodine of iodoprotein. On approximately this latter dosage, she had previously reached the same level of metabolism (fig. 1).

Similar peptides can be prepared by alkaline hydrolysis of the original iodoprotein, although on prolonged hydrolysis these break down into single amino acids, as shown in figure 2. Such mixtures have been tested on 6 patients with hypothyroidism and found to be active. They are reported in a previous paper (4). After partial purification the activity found for this material, in terms of iodine, was about 1/30 to 1/40 that of whole thyroid substance. For comparison, 2 cases of human myxedema were treated with synthetic 3:5 diiodothyronine,[2] as shown in figure 5. Their response is also

[2] This synthetic molecule equals thyroxine minus the two iodines on the benzene ring containing the phenolic group.

approximately 1/30 to 1/40 that of whole thyroid. This order of activity is not far from that obtained in patients by Anderson, Harington and Lyon (7) and by Abderhalden and Wertheimer (8) in animals.

DISCUSSION

The hypothesis that the incorporation of iodine into protein would induce thyroidal activity was first tested nearly half a century ago. However, the classical experiments of Hutchison (9) and of Baumann (10), ending in negative results, discouraged further attempts in this direction for many years. Meantime knowledge of the chemical and physical properties of iodoproteins was advanced through the studies of Blum and Strauss (11) and of Bauer and Strauss (12) without reference to their possible biologic activity. More recently Abelin (13) working with hydrolysates of iodoprotein administered to animals found evidence of hormonal activity. Furthermore, Abelin as well as Ludwig and Mutzenbecher (14) have prepared material resembling thyroxin from such hydrolysates. The observations reported here show that simple iodoprotein as well as its hydrolysates are effective in relieving human myxedema. This evidence that thyroidal activity can arise in a colloidal molecule through simple iodination throws some doubt on the prevailing concept that the thyroid gland manufactures thyroxin in toto and then builds it into a protein for storage. Perhaps in the iodinated colloid itself may be found the beginning of thyroidal activity.

It is interesting that this iodoprotein can be broken down by gentle hydrolysis into active and inactive fractions in the same manner as natural thyroglobulin (15). The activity of iodoprotein is concentrated in the smaller molecules, with the removal of inactive fractions containing diiodotyrosine and other iodinated amino acids. Consequently this finding excludes the possibility that the activity of iodoprotein is due to massive dosage of diiodotyrosine contained therein. The work of Thompson (16) and of Canzanelli and Rapport (17) likewise indicates that diiodotyrosine is too weak in activity to account for the results reported here.

The failure of the older investigators to observe activity in their products can be explained, in part, by the small dosages used, and in part, by the fact that their iodoproteins were derived by methods differing from the one described here. In terms of nitrogen the activity of the iodoprotein is 1/5 that of thyroglobulin; but in terms of iodine it is only 1/400. When inert iodine is eliminated by peptic hydrolysis, the activity of the peptone, in terms of nitrogen approaches one-half that of thyroglobulin. Indeed, since peptic digestion of thyroglobulin usually destroys 3/4 of its activity, the resulting thyroxin peptone, in terms of nitrogen, has about equal potency with the T peptone described here. However, in terms of iodine this peptone is only 1/200 as active as thyroxin peptone. This paradox is explained by the fact that the one has an iodine content of roughly 20% and the other an iodine content of only 0.2%.

When the iodoprotein is split into small fragments, peptides and amino acids, some of the inert iodine present in the T peptone is removed. Conse-

quently the discrepancy in activity on the basis of iodine is reduced to about 1/30 of whole thyroid or thyroxin. The basic reason for this may be the fact that the active amino acid present in iodoprotein is an iodothyronine derivative. Evidence presented above indicates that this order of activity also holds for synthetic 3:5 diiodothyronine.

The production of thyroidal activity by simple iodination of protein raises several problems. (*a*) Does thyronine[3] exist preformed in serum protein as an essential amino acid, awaiting iodination? (*b*) Does the process of iodinating the protein also change molecular configuration so as to produce physiological activity? (*c*) Can the thyroidless organism synthesize iodothyronine molecules from other iodinated residues? Studies of iodinated globin by Cohn, Salter and Ferry (18) suggests that the second question is to be answered in the affirmative. They found that under certain conditions the phenolic groups of diiodotyrosine radicals may disappear when protein is iodinated. The proper interpretation of this chemical process may contribute to a better understanding of thyroid activity.

SUMMARY

These data demonstrate 13 successful responses in myxedema and cretinism and 2 successful responses in thyroidectomized rabbits produced by iodinated serum protein and its acid-insoluble degradation products. This material originated from the brief treatment of blood proteins with compound solution of iodine. In terms of total mass, the activity of the iodoprotein was about 1/5 that of whole thyroid; in terms of iodine, the activity was about 1/400. On the other hand, the acid-insoluble iodopeptone was about half as active as whole thyroid in terms of total mass, but only 1/200 as active in terms of iodine. This discrepancy in activity is due partly to the presence of iodine in inert combination in the iodoprotein and in its derivatives. The low potency in terms of iodine is probably due, also to the fact that the effective iodine-containing group is an iodothyronine derivative, which has lower potency than thyroxin.

Like thyroglobulin, this material can be fractionated with enzymes or by alkaline hydrolysis into thyroxin-like (T) and into diiodotyrosine-like (D) fractions. The latter were inert; the former possessed all the activity present in the original iodoprotein. As with the enzymatic split-products of thyroglobulin, the D fraction yielded no thyroxin-like amino acid after drastic treatment with hot barium hydroxide solution, whereas the T fraction yielded, under such treatment, material which resembled iodothyronine derivatives (related to thyroxin). The latter can likewise be recovered from the original iodoprotein by drastic alkaline hydrolysis without previous separation into component peptones. Such a procedure is the standard method for obtaining thyroxin from thyroglobulin. The activity of this iodothyronine material is of the order of crystalline diiodothyronine, and is roughly 1/30 to 1/40 as active as thyroglobulin in terms of iodine. It corresponds to Abelin's 'homothyroxin' material.

[3] Thyroxin minus all 4 iodine atoms.

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STUDIES ON ANTI-THYROID PRINCIPLES

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WHEN NOVOCAIN or procain is injected subcutaneously in guinea pigs in proper doses, the body temperature is lowered to a considerable degree in one or two hours. Glaubach and Pick (1) have shown that this effect does not occur when thyroxin is administered to the animals for several days prior to the injection of the novocain or procain.

This fact seems to suggest a suitable method for studying the possible antagonistic action of certain substances toward thyroxin. Using another method, one of us (J. B.) with his coworkers (2, 3) has demonstrated that such a protective effect against thyroxin can be obtained with lipoid extracts of blood and pancreas. A similar though less marked effect can be obtained with olive oil and with various preparations of blood manufactured and recommended in Germany for the treatment of hyperthyroidism. The protective action, however, has been noted only in animal experiments. It has not been possible to confirm the therapeutic effect of any of these substances in human beings.

METHOD

A daily subcutaneous injection of 0.2 to 0.3 cc. of thyroxin (Roche) was given for 5 days to guinea pigs of medium size. On the sixth day a 2% solution of novocain or procain was injected subcutaneously in 2 to 3 cc. doses. There was usually no change in the rectal temperature following the administration of novocain or procain; occasionally there was even a slight rise during the first hour.

The substance whose antagonistic action toward thyroxin was to be examined was given simultaneously with or prior to the administration of thyroxin. If novocain or procain caused the temperature of the animal to fall, we felt justified in assuming that an antagonistic action to thyroxin existed. If the temperature showed no marked change, such an antagonistic action was not assumed.

The accompanying table shows that the behavior of guinea pigs is the same in both Vienna and New Orleans, and is also the same whether novocain or procain is used for the test.

RESULTS

A. *Diiodotyrosin*, which has been recommended and is widely used in

[1] From Sombor, Jugoslavia.

Europe for the treatment of hyperthyroidism, if injected in doses of 0.2 to 0.3 cc. of a 2 per mille solution simultaneously with the thyroxin, had no antagonistic action toward it. That is, novocain administered after 5 daily injections of thyroxin plus diiodotyrosin did not lower the body temperature in guinea pigs. Diiodotyrosin injected alone for 5 days had no influence upon the following novocain test; the temperature of one animal, for instance, dropped from 38.4° C. to 35.4° C. within 75 minutes after the novocain injection.

B. Sheep serum uniformly inhibited the action of thyroxin upon the novocain test. It was injected subcutaneously in doses of 0.5 cc. either simultaneously with the thyroxin or for 5 days prior to the injections of thyroxin (that is, 10 to 5 days before the novocain test). In all these experiments procain lowered the body temperature to about the same degree as

TABLE 1

	Rectal temperature (before novocain)	Minimal temperature (after novocain)	Remarks
Unprepared animal	38.3° C.	35.6° C. (after 115 minutes)	
Three injections thyroxin 0.3 cc. Two injections 0.2 cc. prior to novocain test	38.2° C.	37.8° C. (after 60 minutes)	
Unprepared animal	(before procain) 98° F.	(after procain) 94.5° F. (after 60 minutes)	
Two injections 0.3 cc. Three injections 0.2 cc. prior to procain test	100.2° F.	100.0° F. (after 95 minutes)	35 minutes after procain temperature was 102.4° F.

if thyroxin had not been injected. The temperature dropped, for instance, from 38° C. to 34.2° C. in 75 minutes in one experiment, and from 38.2° C. to 35.4° C. in 45 minutes in another.

The effect of sheep serum was the same whether it was obtained from an untreated sheep or from one which had been treated previously with the thyrotropic hormone of the anterior pituitary gland. The treatment with thyrotropic hormone was continued until the sheep serum, when tested on guinea pigs, was shown to possess a definite anti-thyrotropic action. Up to 30 units of thyrotropic hormone were compensated by this serum; that is, up to 30 units of thyrotropic hormone injected at the same time as the serum did not produce the characteristic microscopic changes in the thyroid glands of young guinea pigs under the usual experimental conditions.

C. Human serum was used in the same way and in the same doses as the sheep serum was used in the experiments just described. It also exhibited a definite antagonistic action toward thyroxin in that the temperature of the guinea pigs injected simultaneously with it and with thyroxin dropped considerably following the novocain or procain test. In one instance the tem-

perature dropped from 100.2° F. to 93.8° F. in 60 minutes after the injection of the procain. The administration of human serum prior to the injection of thyroxin has been tried in only one experiment. In this it was only slightly effective, whereas the simultaneous injection of human serum and thyroxin had the same marked effect in all instances.

D. Olive oil injected in doses of 1 cc. simultaneously with thyroxin for 5 days had no apparent effect upon the procain reaction, that is, the temperature of the experimental animal was not markedly lowered.

E. Theelin (Parke, Davis) was injected in doses of 0.005 mg. (50 I.U.) the first, third and fifth days simultaneously with thyroxin which, as usual, was given daily for 5 days. Theelin did not influence the result of the procain test. When injected without thyroxin in the same doses and at the same intervals it also had no effect upon the procain test, and the body temperature fell within the usual time interval and to the usual degree.

F. We have not observed a differential effect upon the procain test following injections of the blood of a normal subject and of two patients with severe hyperthyroidism. Whole blood was injected subcutaneously in guinea pigs in doses of 2 to 3 cc. daily for 5 days. The subsequent injections of procain lowered the body temperature in all instances to approximately the same degree.

SUMMARY

By the use of the method described a principle antagonistic toward thyroxin has been found in sheep serum and human serum. Diiodotyrosin, olive oil and theelin did not exhibit such an effect. The method used in these experiments was not appropriate for the detection of thyroid hormone in excess in the blood of hyperthyroid subjects. Further studies will be made on the effect of injections of blood serum on hyperthyroidism in man.

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THE SOMATIC GROWTH DEPRESSING EFFECT OF TESTOSTERONE PROPIONATE

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IN PREVIOUS communications (1,2) it was pointed out that castration of the male albino rat at 22 days of age led to significant inhibition of general somatic development so that at 80 days of age such animals were both lighter and shorter than their controls. Since deprivation of the male sex hormone incident to castration could result in such marked somatic underdevelopment, it became interesting to see whether the presence of such hormones in excess of normal would have a growth stimulating effect.

Thirty-two animals of Wistar Institute Strain were divided into 2 groups of 16 animals each. Group 1 received daily (except Sunday) intraperitoneal injections of 1 mg. of testosterone propionate (perandren, Ciba) from age 26 to 79 days. Group 2 served as uninjected controls. All animals were otherwise treated similarly being fed Purina Dog Chow and green vegetables. Water was constantly present.

Twenty-four hours after the last injection all animals were killed by bleeding through the carotid artery while under the influence of ether anesthesia. Weekly weights were obtained and final weights and lengths (taken from tip of snout to anus) were determined. All data were subjected to statistical analysis (3), and differences were considered significant only if 'significance ratios' were 3 or more. Graphs were then constructed for weekly weight changes. These graphs were compared with each other and with a graph similarly constructed for animals of the same series which were castrated at 22 days of age (2). In addition, theoretical body weights calculated from observed body length (4) for the two groups were compared with the respective observed body weights in order to determine whether any observed change in length was accompanied by a comparable change in body weight.

RESULTS AND DISCUSSION

From figure 1 it may be seen that after the 50th day of life the animals treated with testosterone propionate became progressively lighter than the normal controls and actually approached the curve previously reported for castrate untreated animals (2).

From table 1, it may be seen that initial body weights of the normal treated animals did not differ significantly from those of the normal controls. At 80 days, however, the treated animals averaged 18.6 gm. lighter and 6.5 mm. shorter than the untreated controls. Both of these differences as may be judged from their respective 'significance ratios' are probably significant.

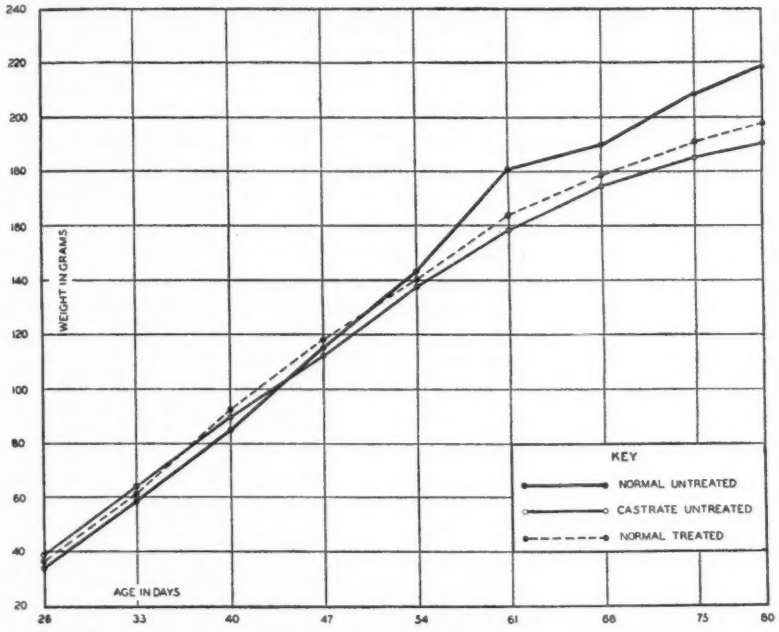

Fig. 1. Comparison of the mean body weights of normal male rats, treated with 1 mg. testosterone propionate daily except Sunday, with normal and castrated untreated animals.

From table 2, which compares the actual or observed final body weights with the theoretical weight calculated according to Donaldson (4), it may be seen that observed body weights of both treated and untreated animals showed differences from the theoretical which were within the limits of random sampling.

In carrying out this work it was found that time could be saved by not injecting the controls with inert substances since from previous experiments (5, 6) it has been shown that such procedures fail to affect the growth curve of such treated animals. It will also be noted that initial body lengths

Table 1. Comparison of initial body weights, final body weights and final body lengths of rats treated with 1 mg. testosterone propionate daily (except Sunday) with their respective control findings

	At 26 days			At 80 days					
	Body wt.	Difference	S.R.	Body wt.	Difference	S.R.	Body length	Difference	S.R.
	gm.			gm.			mm.		
Treated 16 animals	37.8±1.2	1.8±1.8	1.0	199.3±3.1	18.6±4.6	4.1	198.5±1.10	6.5±1.66	3.9
Control 16 animals	36.0±1.4			217.9±3.4			205.0±1.24		

were not determined for the animals but were assumed to be similar for the two groups. Since accurate measurement in a squirming young rat could only be obtained during anesthesia, it was deemed advisable to omit this measurement rather than anesthetize animals which were to be used for growth studies, especially when it is known that body length and weight in the normal rat are so highly correlated (4).

From the data obtained one may conclude that the animals injected with testosterone propionate were significantly stunted in body weight and length. This observation comes as a surprise when one recalls that castration tends toward the same result.

It has been shown by a number of investigations (7–13) that ovarian hormones also inhibit growth but that this inhibition may be rather promptly observed, becoming significant within 4 weeks time (14, 15). The androgens, however, appear to require a longer time to show this effect since Bottomley and Folley (16) failed to note such a depression during a short period of treatment of the guinea pig and McEuen, Selye and Collip (17) reported

TABLE 2. COMPARING ACTUAL FINAL BODY WEIGHTS OF THE CONTROL AND TREATED ANIMALS WITH THEIR RESPECTIVE THEORETICAL VALUES

Control group		Difference	S.R.
Actual body weight gm.	217.9±3.4	6.5±4.8	1.4
Theoretical body weight gm.	211.4±3.4		
Treated group			
Actual body weight gm.	199.3±3.1	10.2±4.4	2.3
Theoretical body weight gm.	189.1±3.1		

a similar non-inhibiting effect in the rat. The latter investigators began injections at 36 to 38 days and killed the animals 23 days later. It may be noted from our growth graphs that the curve of the treated animals just began to diverge away from the normals at about the 24th day of treatment, significant difference being first observed on the 35th day of treatment (61 days of age). Use of the propionic acid ester of testosterone which is more potent (18, 19) than the unattached testosterone used by others (17), in all probability also played a part in the depression noted in the experiments reported here.

The cause of this inhibition is evidently not due to a direct effect of the androgen upon the somatic system since one would expect an increased growth in treated animals, as opposed to inhibition noted in the castrate. In the case of the estrogens, there is sufficient evidence to show that they not only inhibit the gonadotropic function of the pituitary (20, 21) but that they simultaneously depress the somatotropic or growth function (7, 8, 9, 10, 22). It has further been shown that dwarfs resulting from experimental treatment with high doses of estrogens could be made to grow when subsequently treated with anterior pituitary extracts (14, 15). That androgens probably also exert their somatic depression through the pituitary may be gathered

from the fact that testosterone propionate has been shown to act antagonistically to the anterior pituitary (23). Because of this resulting pituitary depression, and because anterior lobe extract can stimulate growth in rats dwarfed by other sex hormones, e.g. estrogens, it is felt that the somatic inhibition noted in these experiments was due to suppression of the growth hormone.

It will be recalled that the final body weights were proportional to the final body lengths for both groups of rats. For the controls, of course, this observation shows how closely to type our own strain of animals is and signifies that the use of Donaldson's formula is valid for our colony. For the treated rats, the lack of significant deviation discloses the fact that while testosterone propionate in the dosage used significantly inhibits body length and weight (table 1) this inhibition is proportional. This observation seems also to indicate that inhibition is probably on a pituitary basis since it is well known that the pituitary dwarf is frequently a well proportioned individual. The proportionate inhibition resulting from this treatment stands in contrast to the disproportionate stunting resulting from castration (1) in which the animals become shorter and lighter but relatively heavy for their body lengths.

Finally, the fact that testosterone propionate in these experiments inhibited body growth leads one to feel that the dosage used was markedly excessive for the maintenance of a normal body economy. Other data to be shortly reported indicate that with smaller dosage such somatic growth depression is lacking. It appears that normal somatic growth is in part dependent upon a proper balance between sex and growth hormones. As long as the one remains within normal limits the other is secreted in amounts sufficient for proper and proportional growth. On the other hand, where, as a result of added sex hormone, the body is flooded, this flooding serves as a check on further growth hormone elaboration. It is as if nature, in its economy stops somatic growth when sex hormone concentration is high enough for procreative purposes. Generally, when in such a state, the body needs no further growth.

SUMMARY

Testosterone propionate given in doses of 1 mg. intraperitoneally daily (except Sunday) from 26 to 80 days significantly depresses body weight and length of the male albino rat.

Depression in weight first becomes noticeable after 24 days of treatment (50th day of life) and then becomes progressively more marked so that the growth curve of such animals approaches the growth curves of rats inhibited by castration.

In contrast to the disproportionate growth inhibition of the castrate male which becomes shorter and lighter but relatively heavy for its stunted length, animals treated with testosterone propionate become proportionately inhibited.

It is felt that dosage and time are important factors in the production of the observed effect.

The mechanism of action is discussed and it is felt that testosterone propionate in the dosage used, like estrogens in high dosage, exerts its growth depressing effect by inhibiting growth hormone production of the pituitary gland.

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A RAPID EXTRACTOR FOR URINARY ANDROGENS

FACTORS TO BE CONSIDERED IN THE PREPARATION OF EXTRACTS FOR COLORIMETRIC ASSAY[1]

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THE PURPOSES of this communication are, first, to describe a modified extraction apparatus, and second, to show that a significant loss of androgenic hormones occurs in the preparation of extracts for colorimetric assay.

The current literature contains numerous reports on various methods and types of apparatus for extracting urinary hormones. All of these require two or more hours for extraction. The apparatus described by Gallagher, Koch and Dorfman (1) is, so far as we know, the most rapid one. The space required, however, makes it too cumbersome for the average clinical laboratory. The apparatus described here is conveniently small and permits extraction of about 90% of the androgenic hormones from 1 liter of urine in 1 hour.

During the course of testing the apparatus for completeness of extraction it was discovered that much less than 90% of a known amount of added synthetic androgen could be recovered even if periods of extraction longer than 1 hour were employed. It was found that the chief loss of hormone could be attributed to the treatment of extracts with decolorizing charcoal. The data which led to this conclusion are reported in detail. The results are expressed in terms of Callow's (2) 'sterone' milligrams to indicate clearly that these are chemically defined units and do not correspond to the biologically defined international units.

APPARATUS

The apparatus[2] (fig. 1) is a modification of the continuous benzene extractor described by Smith and Smith (3). The chief innovation is the glass stirrer which serves to break up the benzene into multiple small droplets and thereby markedly increases the surface area of the benzene as it passes up through the urine. Wang and Wu (4) have also employed mechanical stirring in an extraction apparatus. Their apparatus, however, is complicated and cumbersome. Moreover, they did not make accurate recovery experiments.

[1] This work was aided in part by grants from the Rockefeller Foundation, administered by Professors F. L. Hisaw and G. R. Harrison.

[2] Macalaster Bicknell Company, Boston, cooperated in the design and manufacture of this apparatus.

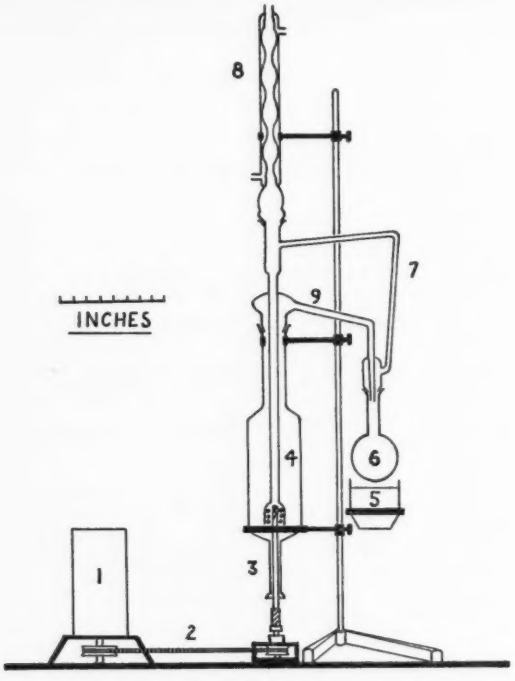

Fig. 1. Description of apparatus

1) Induction motor, rated at 1200 RPM, connected to 3″ pulley.
2) Belt.
3) Jena CPG all glass stirrer which is ground to fit into glass sleeve. The stirring rod has a slight twist at its upper end. It is connected to the 1½″ motor driven pulley by means of a short length of rubber tubing. The tubing is fitted over a detachable adaptor at its lower end so that the stirring rod and rubber tubing can be disconnected from the pulley. The stirrer revolves at about 2000 RPM. No lubrication other than the urine is required.
4) Flask for urine. One liter of urine fills the wide lower section of the flask. The neck is filled with benzene. The tube running down through the middle of the flask has a small inverted bell at the bottom end. This is drilled with about twelve 0.25 cm. holes. The stirrer revolves within the bell and forces small droplets of benzene through the holes.
5) Adjustable electric hot plate which heats a Wood's metal bath.
6) 250 cc. flask for benzene.
7) Asbestos lagged tube through which benzene vapors pass.
8) Water condensor. This condenses the benzene vapors. The benzene fluid drops down the tube to bottom of flask 4.
9) Tube for return flow of benzene from urine flask to benzene flask.

METHODS[3]

The method of hydrolysis and purification of extracts for colorimetric assay is similar to that described by Oesting (5). The essential points are outlined below.

[3] No grease or oil was used in the apparatus because it was found that these give a small but appreciable color at 5200 Å. The apparatus was glass throughout to avoid contamination of the extracts with rubber or cork materials. The ether was freshly distilled. All reagents were of Merck's 'reagent' quality. The time of shaking the ether extract with NaOH and water was standardized at 30 seconds. After shaking, 2 minutes were allowed for the separation of the ether-NaOH emulsion, and 2, 5 and 10 minutes for the 3 water washings respectively. Solutions were transferred quantitatively from one con-

Urine, collected without preservative.

A ↓

Hydrolysed by boiling 10′ after adding 15 vol.% commercial HCl. Cooled under tap water after hydrolysis.

B ↓

Extracted in continuous benzene extractor (fig. 1).

C ↓

Benzene (flask 6, fig. 1) distilled off over boiling water bath, residue transferred to separatory funnel with 1 50 cc. and 2 25 cc. portions of ethyl ether.

D ↓

Ether washed 5 times with 25 cc. portions 10% NaOH followed by 3 washings with 30 cc. portions distilled H_2O. Washings discarded. Ether transferred to beaker. Separatory funnel rinsed with 50 cc. fresh ether. This saved for washing charcoal in next step.

E ↓

Ether stirred with 0.50 gm. decolorizing charcoal (Norite A) (Pfanstiehl), filtered through Whatman #50 paper with aid of suction. Charcoal washed once with 50 cc. fresh ether (see preceding step).

F ↓

Ether distilled off over water bath. Residue taken up in 2 to 4 cc. absolute alcohol.

G ↓

The absolute solution was assayed for its "sterone" content by the procedure reported (6, 7).

RESULTS

Rate of extraction. The rate of extraction was determined in 2 experiments by extracting 1 liter of urine over 3 consecutive hourly intervals. It was assumed that all the extracted hormone was contained in the benzene flask (6, fig. 1). The results (table 1) show that over 90% of the 'sterone' substances which could be extracted in 3 hours were recovered from the

TABLE 1. RATE OF EXTRACTION

Substance	No.	Time, minutes	'Sterone,' mg.	% of total material extracted
Urine A	1	0 to 60	15.4	91
	2	60 to 120	1.0	6
	3	120 to 180	0.6	3
Urine B	217	0 to 60	9.2	93
	218	60 to 120	0.4	4
	29	120 to 180	0.3	3

urine during the first hour. Therefore, extraction over a period of 1 hour should suffice for most experimental purposes provided conditions are maintained constant.

Recovery experiments. In order to test the efficiency of the whole extraction and preparation procedure, recovery experiments were done. In these a weighed amount of synthetic androgen was added to half of a sample of urine before extraction (Step B in outline). Both halves were assayed for 'sterone' content. It was found (see below) that recovery was less than expected. Furthermore, experiments showed that the loss was independent of the duration of the extraction procedure. It was thought, therefore, that the loss occurred after extraction. This possibility was investigated by recovery experiments at each step in the procedure starting at Step G and working backwards to Step A. Androsterone and dehydroisoandrosterone were the androgens employed.

The data (table 2) are arranged to indicate the step at which the synthetic

tainer to another. The charcoal was stirred for a measured period of time, at first by hand, later with a mechanical stirrer (see under data). Care was taken that no loss occurred from foaming or bumping during evaporation of solvents. Care must be taken to have glassware chemically clean.

TABLE 2. RECOVERY EXPERIMENTS (see footnote for key)

Androsterone

Step	Substance	Test	Exp. No.	Desc.	Mg. observed	Rec.	K.	R.	Av.
G	Final ur. ext.	M. of meas.	214A	ur. ext. (1 cc.)	1.90				
			214C	1 cc. 214A plus 0.85 mg. A.	2.8	0.9	.85	1.06	
F	Ur.	(*a*) M. of meas.	244A	ur. ext. (45 cc.)	1.1				
		(*b*) Loss in post-ch. tr.	246A	ur. ext. (45 cc.) plus 2.3 mg. A after ch.	3.3	2.2	2.3	.96	
			248A	ur. ext. (38 cc.) plus 3.9 mg. A after ch.	4.6	3.7	3.9	.94	
			250A	ur. ext. (38 cc.) plus 6.3 mg. A after ch.	6.8	5.9	6.3	.94	
									.95
E_1	Syn. A in ether	Loss in ch. tr.	231	2 mg. A −3′ ch.	1.1		2.0	.55	
			232	4 mg. A −3′ ch.	2.5		4.0	.62	
			233	8 mg. A −3′ ch.	3.6		8.0	.45	
									.54
E_2	Syn. A in ether	(*a*) Loss in ch. tr.	236	.95 mg. A no ch.	1.0		.95	1.05	
		(*b*) Time of tr. effect	237	.95 mg. A −3′ ch.	.55		.95	.58	
			238	.95 mg. A −8′ ch.	.57		.95	.60	
			239	.95 mg. A −15′ ch.	.53		.95	.56	
									.58
E_3	Final ur.	Loss in	228	3 cc. final ext. −3′ ch.	3.0				
			229	3 cc. final ext. +5.95 mg. A −3′ ch.	6.8	3.8	5.95	.64	
E_4	Ur.	Loss in ch. tr.	244	45 cc. ether−3′ ch.	1.1				
			245	38 cc. ether+ 1.85 mg. A−3′ ch.	2.0	1.1	1.85	.60	
			247	38 cc. ether+ 4.0 mg. A−3′ ch.	3.7	2.8	4.0	.70	
			249	38 cc. ether+ 6.6 mg. A−3′ ch.	5.8	4.9	6.6	.74	
									.68
D_1	Ur.	Loss in sep. and ch. pro.	216A	½ ether−3′ ch.	3.60				
			216B	½ ether+3 mg. A−3′ ch.	6.00	2.4	3.0	.80	
D_2	Ur.	Sep. pro.	224	Ext. of ether w. 223.	0.40				
			226	Ext. of ether w. 225.	0.26				
B_1	Ur.	Com. pro. 1 hr. ext.	225	1 L. ur.−3′ ch.	6.4				
			223	1 L. ur.+6 mg. A−3′ ch.	11.2	4.8	6.0	.80	

TABLE 2.—*Continued*

Step	Substance	Test	Exp. No.	Desc.	Mg. observed	Rec.	K.	R.	Av.
Androsterone									
B_2	Ur.	Com. pro. 1 hr. ext.	220B	1 L. ur.−3′ ch.	6.6				
			220A	1 L. ur.+4 mg. A−3′ ch.	9.0	2.4	4.0	.60	
			221B	1 L. ur.−3′ ch.	6.6				
			221A	1 L. ur.+4 mg. A−3′ ch.	10.4	3.8	4.0	.95	
B_3	Ur.	Com. pro. 1. hr. ext.	214A	1 L. ur.	7.6				
			214B	1 L. ur.+6.0 mg. A	11.0	3.4	6.0	.57	
B_4	Ur.	Com. pro. 2 hr. ext.	A	1 L. ur.	6.8				
			B	1 L. ur.+4 mg. A.	8.4	1.6	4.0	.40	
B_5	Ur.	Com. pro. 1 hr. ext.	A	1 L. ur.	5.8				
			B	1 L. ur+2 mg. A.	8.2	2.4	3.7	.65	
Dehydroisoandrosterone									
E_5	Syn. DA in ether	Loss in ch. tr.	242	3.7 mg. DA−no ch.	4.0		3.7	1.08	
			240	3.7 mg. DA−3′ ch.	1.46		3.7	0.39	
			241	3.7 mg. DA−3′ ch.	1.1		3.7	0.30	
									0.35
E_1	Final ext. ur. +DA.	Loss in ch. tr.	228	3 cc. ext.−3′ ch.	3.0				
			227	3 cc. ext.+5.7 mg. DA−3′ ch.	5.2	2.2	5.7	0.39	
B_8	Ur.+DA.	Loss in whole pro. 1 hr. ext.	222B	1 L. ur.	6.8				
			222A	1 L. ur.+4 mg. DA.	8.0	1.2	4.0	0.30	
									.35

Key to Table 2

A	Androsterone	ext.	extract	pro.	procedure
Av.	average	hr.	hour	R.	ratio
ch.	charcoal	K.	known	Rec.	recovered
com.	complete	L.	liter	sep.	separation
DA	dehydroisoandrosterone	meas.	measurement	tr.	treatment
Desc.	description	M.	method	ur.	urine
exp.	experiment	mg.	milligrams	w.	washings

hormone was added. The ratio at right represents $\frac{\text{mg. 'sterone' recovered}}{\text{mg. 'sterone' added}}$.

A ratio of 1.0 indicates complete recovery. The experimental error of the spectro-chemical (colorimetric) 'assay' is estimated at $\pm 10\%$. The data obtained show the following facts.

(*a*) Androsterone added to the final urine extract (Step G) or to the ether extract after decolorizing charcoal treatment (Step F) is completely recovered.

The determinations made by this spectro-chemical 'assay' method agree within $\pm 6\%$ of the known values.

(*b*) Androsterone added to the ether extract before decolorizing charcoal treatment (Step E) is not completely recovered. The average loss of androsterone in pure ether stirred with charcoal (mechanical stirrer) for 3 minutes is 46% (E_1). This loss is essentially constant for amounts of androsterone ranging from two to eight mg. The loss of dehydroisoandrosterone[4] in the charcoal procedure is greater (65%, E_5). No loss was observed when ether solutions of hormones were carried through the procedure without charcoal treatment (E_2), nor was the loss of androsterone greater if the time of charcoal treatment was prolonged from 3 to 8 or 15 minutes (E_2). Loss of androgen from final urine extract residues (E_2E_6) in ether was essentially the same as for pure solutions of androgen in ether. The average loss from untreated urine extracts (E_4) was slightly less (32%) than for pure solutions (43%, $E_1E_2E_3$).

These facts lead to the following conclusions. Decolorizing charcoal is responsible for a significant loss of androgen in ether solutions. The loss of dehydroisoandrosterone is significantly greater than the loss of androsterone. The loss of hormone from crude urine ether extracts is slightly less than from pure ether solutions of androgen.

(*c*) Loss in the separation of alkali-soluble substances from the neutral substances in urine extracts is small. The loss of androsterone added at Step D approximated the loss from charcoal treatment. The ether washings (NaOH, H_2O) were re-extracted with ether in one experiment (D_2) and the 'sterone' content estimated. The 'sterone' recovered from the washings represented less than 4% of the total amount recovered in the whole procedure.

(*d*) Loss of hormone in the complete procedure (34%) (Step B) was essentially the same as the loss due to the charcoal treatment of urine extracts (32%, E_4). The variation in results obtained in the complete procedure may be due to the fact that the ether-charcoal mixture was stirred by hand rather than by the mechanical stirrer later adopted and to the fact that the period of charcoal treatment was timed only in experiments B_1 and B_2.

SUMMARY OF RESULTS, ANDROSTERONE

	Recovery	
	range %	average %
Loss in complete procedure	40–95	66
Loss in separation (Step D through E)	small	(about 4%)
Loss in charcoal treatment:		
Urines	60–74	67
Final extracts		64
Synthetic solutions	45–62	56

The results of tables 1 and 2 lead to the impression that about 90% of the 'sterone' content of hydrolyzed urine is extracted during the first hour of extraction and that the decolorizing charcoal employed to clear urine extracts for colorimetric assay removes a significant amount of androsterone and dehydroisoandrosterone from the extracts.

[4] It has been shown in another communication (7) that dehydroisoandrosterone and androsterone behave identically in the m-dinitrobenzene reaction.

DISCUSSION

Evidence has been presented to show that with the modified Smith and Smith (3) apparatus described above, about 90% of the 'sterone' content of hydrolyzed urine may be extracted with benzene in a period of 1 hour. The high efficiency of this apparatus is attributed to the mechanical stirrer which breaks up the benzene into fine particles before it passes through the urine, and to the rapid circulation of benzene obtained by the use of Wood's metal bath. In the original Smith and Smith apparatus which had no stirrer, from 12 to 24 hours were required for complete extraction.

Oesting (5) in 1937 described a procedure for the colorimetric assay of male sex hormone in the urine. His procedure included decolorization of extracts with adsorbant charcoal. From unpublished data based on biological methods of assay he concluded that the charcoal caused no significant loss in the androgenic hormone content of his extracts. Callow et al. (2) apparently confirmed his finding although they did not publish supporting data. The present investigation shows that under the conditions used by us the decolorizing charcoal procedure removes a significant amount of androsterone and dehydroisoandrosterone from pure ether solutions and from ether solutions of urine extracts. Biological methods of assay are probably not sufficiently sensitive to detect loss of androgenic hormone especially if a major part of the androgen lost is dehydroandrosterone because that hormone has only one-third the biological activity of androsterone.

Using a colorimetric method Neustadt (8) has reported recovery experiments in which he used a different method of extraction but the same purification procedure. In 4 out of 7 experiments approximately 100% of the added amount of androsterone was recovered. Unfortunately, he added only small amounts of the androgen. In recovery experiments, a relatively large amount of substance compared to the amount already present in the sample should be added. Otherwise the experimental errors in the determined recoveries do not permit of any accurate interpretation of the results. It is possible that had Neustadt added larger quantities of the hormone, he might have detected the loss which is apparent in our data. It is also conceivable that his decolorizing charcoal behaved differently from ours.

The data at hand are insufficient to permit us to state whether the loss is consistent or to offer a correction factor. It can be stated, however, that the loss is important and that it should be considered whenever urine extracts are decolorized with charcoal.

SUMMARY

A small and convenient apparatus which permits extraction of about 90% of urinary androgens in 1 hour with benzene is described.

Recovery experiments made in part with the aid of this apparatus show that a significant amount of androsterone and of dehydroisoandrosterone is lost in the preparation of urine extracts for colorimetric assay. The chief loss occurs when the ether extract is cleared with decolorizing charcoal.

The facts presented led to the conclusion that the amount of androgenic

'sterone' substances determined by the usual colorimetric methods which include treatment with decolorizing charcoal as part of the procedure are not an accurate index of the original 'sterone' content of the urine.

Dr. Erwin Schwenk of the Schering Corporation generously supplied the synthetic androsterone and dehydroisoandrosterone used in these experiments.

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RESPONSES OF THE FEMALE TO MALE HORMONE SUBSTANCES

WITH NOTES ON THE BEHAVIOR OF HENS AND NEWLY-HATCHED FEMALE CHICKS[1]

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THERE IS an accumulating body of evidence that the female organism is capable of producing male hormone (androgenic) substances and of reacting morphologically and physiologically to these substances.

The production of androgenic substances by the female under normal conditions. Androgenically active material is excreted daily in the urine of normal women. The amount has been calculated to be from 42 to 56 international units (I.U.) (1). Although these figures cannot be considered as final values they serve to indicate that women excrete appreciable amounts of androgenic material, in terms of activity, as much as two-thirds of that excreted by men. Callow and Callow (2) have isolated from the urine of normal women, androsterone and dehydroandrosterone, which are the two androgenic compounds known to be present in the urine of men; moreover, the yield was comparable to that obtained from the urine of men. It is entirely possible that these two androgenic end-products in the urine of women are derived from a different source from those of the male, but the point to be emphasized is that there are female hormones in the male individual and that male hormones are present in sizable quantities in the body tissues and fluids of the female individual. Sex is, to some extent, a matter of quantitative hormone dominance and the capacity of the individual to respond to different substances.

Females of species other than primates also show evidence of the presence of androgens. Perhaps the most familiar example is the hen. The comb grows in response to androgens but not to female hormones such as estrone. Thus the well-developed comb of the hen is indicative of the production and utilization of androgenic material. In rats the preputial and paraurethral (3, 4) glands of both males and females exhibit marked growth in response to androgenic but not to estrogenic or luteal hormones. The response of these glands in the female rat to androgens only, suggests that the partially developed state of these glands in the normal female rats may be due possibly to androgenic material. In various strains of female rats the percentage incidence of paraurethral glands was, respectively, 9.4, 26.0 and 8.7 (4, 5). Witschi, Mahoney and Riley (5) by selective breeding have established a strain of rats in which 'prostate lobes' appear in 77.3% of the females, but the greater incidence may be due to the reactivity of the animal rather than to any increased production of hormone substance.

The production of androgenic substances by the female under experimental and

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pathological conditions. In addition to the evidence that androgens are present in the normal female there is experimental proof that both the ovary and the adrenal can participate in the production of androgens (6). Androgens have been obtained from crude extracts of guinea pig ovaries (6). Injection of APL material in female rats stimulates the ovaries, and under certain experimental conditions, there appear histological changes (7) in the anterior pituitary which can be simulated by administration of male hormone substances. This is suggestive that ovaries in situ may produce an androgen-like substance.

By irradiation of the ovary or by the use of anterior pituitary lobe or APL extracts, the female guinea pig may become masculinized, presumably from endogenous hormones (9–12). A similar state of masculinization can be induced in female offspring by a certain mating (♂ *Cavia aperea* × ♀ *Cavia cobaya*).

Recent work (14, 15, 16) with mice and with rats (17) has served to delineate some of the ovary's capacity for the production of androgens, a capacity indicated earlier by Lipschütz (18). When ovaries were transplanted to the ears of castrated males they tend to prevent assumption of a castrate condition by the prostate and seminal vesicles. Deanesly points out that such an androgenic action is associated with luteinization of the theca interna of follicles in the grafted ovaries. She also states that the amount of androgenic activity is slight since the seminal vesicles, for whose growth more hormone is required than for the prostate, are less developed than the prostate. Hill states that the temperature of the ear, which is lower than that of the abdomen, is responsible for the androgenic activity of ovarian graft in the mouse; Deanesly did not confirm this in the rat. Both Hill and Deanesly found that administration of gonadotropic substances does not result in a greater output of androgenically active material by the graft.

The adrenal may also be involved in the production of male hormone substances. Women with hyperplasia or tumor of the adrenal may exhibit a masculine or virilism syndrome (19). Callow (20) has suggested that dehydroisoandrosterone, an androgenic constituent of normal urine may have its origin in the adrenal. Androgenically active material has been isolated from adrenal glands (21), from the urine (20) of normal women and from a female with an adrenal tumor. Crystalline desoxycorticosterone besides exhibiting a cortin-like capacity has androgenic properties, which amount to 0.4 I.U. per mg. of desoxycorticosterone acetate (22).

The response of the female to male hormone substances. (*A*) *Morphological.* That the normal female may respond to androgenic material present in the body is exemplified by growth of the hen's comb. When large amounts of male hormones are administered to female rats, marked growth occurs in those structures which are reactive to androgens, especially in the clitoris (23), preputial and paraurethral glands (3, 4). In addition muscular growth and mucous secretion are induced in the uterine cavity (24) and the cell constituents of the pituitary may come to resemble those of the male in type and in number (25). If exposed to large amounts of male hormone substances at a critical period in embryonic life the female becomes a pseudo-hermaphrodite and appears externally as a male (26–30). There may be a scrotum and instead of a separate perineal vaginal orifice a male type of common urogenital system. Nipples are lacking in embryonically-masculinized females of species in which the males have no nipples. Other details of a similar nature are described by Witschi (31) and by Willier (32).

(*B*) *Physiological.* Besides morphological changes, cyclic reproductive function of the female is interrupted (33) and certain behavioral phenomena of a male type are apparent. Testis grafts may change the behavior of the female guinea pig to a more masculine type (34, 35). A similar observation has been made following the use of crystalline male hormone substance in female rats (36).

Androgens induce crowing (37) and other masculine behavior in newly-hatched male chicks. Since the behavior of the rooster and hen are normally different and very distinctive, adult hens and newly-hatched female chicks were selected as the test animals for study of the capacity of the female to react in a masculine manner to synthetic male hormones.

MATERIAL AND METHODS

Hens. Twenty-five adult hens, bred from Plymouth Rock females and Rhode Island Red males, were used when they were 257 days of age. A few of the birds had commenced laying several weeks before the experiment was begun. Some of the birds had received no treatment prior to this study, others had received a subcutaneous injection of 5 or 20 mg. of testosterone propionate at the time of hatching. Since the responses now under study were obtained from both previously treated and untreated birds, the treatment employed at hatching will not be described further.

Male hormone substance, testosterone propionate,[2] was given either as a 180 mg. pellet or as intramuscular injections on alternate days of 4.0 mg. in 0.1 cc. of peanut oil. Five birds received the hormone in the form of pellets implanted subcutaneously, 6 birds as subcutaneous injections in peanut oil. After 56 days of treatment, hormone injections were discontinued and pellets removed.

The weight and comb growth of each bird had been followed from hatching. The measure of comb growth was expressed as the sum of the length, width and height in mm. Direct measurement was made with a mm. rule. Obviously this is not a true expression of comb volume and a small increase in these measurements represents a large increase in the volume of the comb.

Behavior considered as masculine included crowing and treading, possibly to some extent posture, carriage and a low, husky vocal sound. Observations were made of a tendency toward wing flapping, fighting or the assumption of a high perch, when these much exceeded the degree seen in control animals. Inclusion of these actions as masculine traits is questionable.

Crowing was acceptable only when it appeared as movement culminating in arching and extension of the neck with ruffling of the neck feathers, opening of the bill and emission of a strident lengthy vocalization (fig. 2).

Two runways of different height were provided and observations made of the posture, carriage of the head and tendency of birds to seek a high place, especially while engaged in crowing.

Fighting was recognized when 2 birds jumped into the air to attack each other by striking with the inner, or 'spur', side of the feet. During this attack the birds circled about, facing each other with head erect, neck feathers and wings outstretched. It must be remembered, however, that normal hens fight in this manner.

Pecking at other hens and aggressiveness in forcing away other birds were not considered as cock-like behavior, but were noted.

Moving picture and sound records were taken of the crowing and other behavior. In this way more direct comparison could be made of the effect of

[2] Testosterone propionate and androsterone were furnished through the courtesy of the Ciba Pharmaceutical Company, Inc., the former compound under the trade name perandren.

different types of treatment on the same bird. The hens were kept together in a flock. They were under direct observation for not more than an hour each morning. An attendant who was within a few feet of the flock from 7 A.M. to 12 noon and from 1 P.M. until 4 P.M. also kept a record of the birds which crowed during this time.

One hen (225) which did the most vigorous crowing of all the hens receiving testosterone propionate subsequently received a second treatment with androgenic material. Twenty-three days after the removal of the testosterone propionate pellet, 253 mg. of androsterone were implanted in the same form of pellet used for testosterone propionate.

Chicks. Observations were made of newly-hatched chicks which received testosterone propionate for other experiments. In this manner there were available 43 females of the same breed as the hens described above and 32 female White Leghorns. All received large amounts of testosterone propionate in the from of crystals (38) (5 to 20 mg.), pellets (15 mg.) or oil solutions (37) (0.5 to 2 mg. daily) in such a manner as to be adequate for inciting crowing in male chicks of the same age. Hormone administration was begun within a day after hatching except in 9 White Leghorns which received no treatment until a week after hatching. The masculine behavior (37) of the newly-hatched chick that is reported below is confined to crowing since this action is clear-cut and probably indisputable.

RESULTS

Hens. Comb growth was evident within 2 days after the beginning of treatment. Figure 1 shows the average comb size of the different groups of chickens during the first 30 days of treatment. The comb became red and turgid but did not increase to a size as large as is seen following similar treatment of White Leghorn hens. No direct correlation in the injected birds was seen between the amount of crowing and the amount of comb growth as measured by this method. A few control hens had combs practically as large as experimental animals yet were never seen to crow. *Hen 225*, which was observed to crow approximately 20 to 200 times as frequently as other hens, had a comb smaller than that of several of the treated hens which were not observed to crow.

Since the birds were not under continuous observation crowing may have occurred earlier than in the first recorded instance which was after 6 days of treatment and may have been done by some of the 8 androgen-treated birds which were not seen to crow. In a subsequent similar study of hens under continuous 24-hour observation, crowing occurred commonly only in the early morning. Thus, since the other 8 injected birds were not watched at dawn and shortly thereafter unrecorded crowing might have occurred. Only in *hen 225* was crowing an outstanding and persistent feature throughout the day, although other birds of the same age received pellets of the same size. Usually *hen 225* gave one or 2 daily series of from 2 to 19 crows. Hens 84 and 67 were also seen to crow. In all 3 birds a series of a half dozen or more calls were commonly given in quick succession whenever crowing

occurred. In 5 periods of 2 minutes each, *hen 225* was observed to crow respectively; 10, 7, 9, 6 and 11 times.

As shown in figure 2, the action of crowing resembles that in the cock. The neck is arched and the neck feathers ruffled. The neck is elevated and arched in an S-shape, the head projected forward, the bill opened. Vocalization occurs for 2 or more seconds. The call is harsh and strident, usually modulated like that of a rooster. The same hen commonly employed a dif-

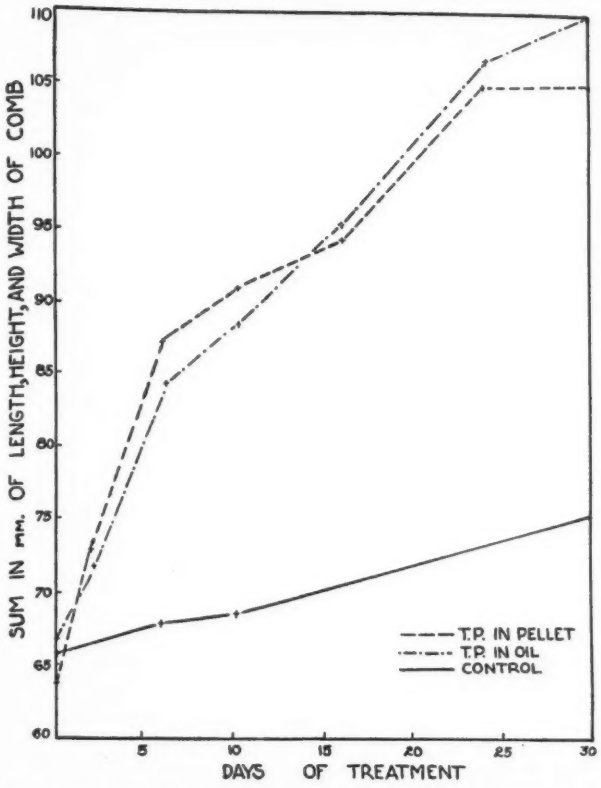

Fig. 1. This shows the average amount of comb growth for the different groups of birds. Among the injected birds the amount of comb growth of the individual hens was not proportional to the amount of crowing.

ferent set of pitches from call to call, especially in the latter ones of a series which were frequently short and incomplete. These abbreviated vocalizations generally omit the first parts of the call. In *hen 225* which afforded the best opportunities for observations, the occurrence of such incomplete calls was restricted chiefly to periods at the beginning and after cessation of hormone administration.

No intermediate stages were seen in the development of crowing such as would be comparable to that of the short high-pitched squeaky crow heard

in the newly-hatched male (37) or female chick injected with testosterone propionate. From a behavior devoid of crowing the hens assumed a fairly adult type of loud crowing without passing through the infantile type of crowing behavior. The early crows were somewhat higher in pitch and had a tendency to 'break,' but all vocalizations were loud, strident and long.

Wing flapping was the same sort of expression reported in the androgen-injected chicks (37). The wings were flapped up and down generally 2 or 3 times, occasionally, as many as 5 times in succession. There was little difference from the type of flapping done by control hens, but the frequency of flapping appeared to be greater.

Attempts at treading or mounting of other females were never observed, an outstanding and obvious void in the approach toward assumption of what had been accepted as a male pattern of behavior.

There seemed to be an increased tendency of the injected hens to fight and peck at other hens. It is questionable, however, whether or not an increase in pecking should be regarded as male behavior. While receiving

TABLE 1. THE NUMBER OF MG. ABSORBED FROM THE PELLET OF TESTOSTERONE PROPIONATE IS NOT RELATED PROGRESSIVELY TO THE AMOUNT OF CROWING EXHIBITED. SIMILARLY CROWING WAS SEEN IN ONLY ONE OF 6 HENS, EACH OF WHICH RECEIVED SUBCUTANEOUS INJECTIONS IN OIL SOLUTION OF 112 MG. OF TESTOSTERONE PROPIONATE.

No. of bird	Weight of pellet in mg.		Mg. injected subcutaneously in oil solution	Mg. of testosterone propionate absorbed	Crowing
	At implanation	At withdrawal 51 days later			
76	180	128		52	−
71	180	85		95	−
84	180	114.5		62.5	+
204	180	116		64	−
225	180	86		94	+++++
67			112		++
90			112		−
100			112		−
70			112		−
202			112		−
11			112		−

hormone some of the hens came to dominate control hens whom they had previously avoided. Such domination had been seen in that a hen of inferior rank would not approach a dominant hen and would run away when a dominant hen came in her vicinity. Occasionally the dominant hen would accelerate the departure of the other hen by administering a vigorous peck. The increased domination shown by treated hens was far from complete, however, and the 3 ruling hens of the 25 in the flock were uninjected controls who had dominated the others before injections were begun.

Weight was not a factor in this matter of supremacy. Individual birds had a range of from 1927 to 2974 gm., and prior to the period of injections had been separated into control and experimental groups in such a manner as to place birds of equal weight in each.

The quantity of administered androgen could be related only roughly to the amount of crowing. Neither did the weight in mg. of testosterone propionate absorbed from the pellets bear any progressive relationship to

the amount of crowing (table 1). Crowing hens, *225* and *84*, absorbed only about two-thirds the amount absorbed by *hen 71* which did not crow. Moreover, the pellets of crowing and non-crowing hens had been made in the same mould and were of the same size and weight and were implanted in a similar site. Subcutaneous injection of a total of 116 mg. of testosterone

Fig. 2. Enlargement of a frame from 16 mm. moving picture film showing the cock-like crowing behavior induced in the adult hen upon treatment with male hormone substance.
Fig. 3. Crowing in a white leghorn chick.

propionate in doses of 4 mg. in oil on alternate days induced crowing in *hen 67*, but in none of the other birds receiving the same treatment. It is of interest that the lesser amounts of hormone given in pellet form were as effective as the greater amounts given in oil solution.

After the removal of the pellet of testosterone propionate the crowing

activity of the hens was lessened and when some of them were placed in separate cages they were observed to lay eggs again. Upon killing 2 months later, 7 of the birds, which had received androgens, were found to have functional reproductive organs and eggs in various stages of development.

One hen, *225*, which had crowed most frequently, was observed to lay eggs within 3 weeks after the pellet of testosterone propionate had been removed. She was then given a 253 mg. pellet of androsterone. She began to crow as vigorously and persistently as when carrying the pellet of tes-

FIG. 4. CROWING BEHAVIOR MAY BE INDUCED BEFORE COMB GROWTH IS APPRECIABLE. Note ruffling of feathers on the back of the neck.

tosterone propionate and 2 days after implantation of the pellet stopped laying. Moving picture records of the crowing showed that the manner of crowing was the same with either androgen. After the pellet was removed, she began laying eggs but continued to crow for at least 6 weeks.

Chicks. The rate of comb growth in chicks was rapid and similar to that tabulated previously (38). The color of the combs became yellow-orange, finally red. Crowing appeared before the combs had grown appreciably and while their color was still yellow. Some animals crowed within 36 hours after injection when the animals were only 2 days old. No correlation could be established between the rate of comb growth and the time when crowing appeared, and none of the chicks which crowed had a comb larger than that seen in birds of the same breed which did not crow.

The act of crowing resembled that exhibited by androgen-treated males of the same age (37). With limited photographic equipment it was difficult to obtain good pictures of the rapid movements, but figure 3 of a female White Leghorn shows a side view with neck feathers ruffled. Figure 4 of a hybird male may be viewed in comparison and as an illustration of the assumption of crowing behavior before the stimulation of comb growth is appreciable.

In contrast to the male chicks, only a few of the females were observed to crow, and these did so with less vigor. Only 6 of the hybrid females and 4 of the White Leghorns were observed to crow but it must be remembered that the observations were not made longer than an hour a day. Of an equal number of male chicks over 3 times this number were seen to crowing during roughly comparable periods of observation.

Crowing was observed within 36 hours after the pellets were implanted in day-old chicks and was of such a short duration and accompanied by such brief movements that it was often difficult to distinguish which chick had crowed. From 3 to 10 days following administration of hormone, the movements and vocalizations were more pronounced and of slightly longer duration. Even so, the whole sequence of events was brief, usually lasting about one second, but was repeated by certain birds with great frequency. The vocalization was a short, feeble, high-pitched squeak, uttered in a monotone.

The female chicks, like the male chicks, with one possible exception did not develop an adult type of vocalization. Instead both males and females later ceased all crowing, even though the administration of the large amounts of hormone was continued and the comb showed an obviously continuing response to the androgen. In the males crowing commonly ceased after from the third to the ninth week, and in the females frequently after the first week and almost completely by the fourth week. Disappearance of crowing behavior generally occurred later in the White Leghorn than in the hybrid chicks. Limits of the time for the cessation of crowing were very variable and would perhaps be changed under different experimental conditions or modified if a larger number or different kinds of birds were used, but the principle that despite continued presence of androgenic material crowing ultimately ceased, was kept inviolate by these chicks which were under study in small groups over a period of 8 different months throughout the year. This cessation of crowing appears puzzling in view of the stimulation of crowing reactions in adult hens and capons (39) after administration of the same hormone and must undergo further study before the phenomena can be accepted as general.

DISCUSSION

Hens adopted a crowing behavior not by mimicking the cockerel, but like newly-hatched chicks, and were able to conduct this complex procedure without benefit of previous experience or of example. It seems logical to conclude that at least a certain number of female chicks and chickens may possess a characteristic pattern of male behavior which can be abruptly assumed upon stimulation of the animal by effective levels of androgenic

substances. Upon removal of the androgenic material the degree of crowing behavior is usually diminished.

It would seem that the crowing response in any individual bird may be dependent to some large extent upon factors in the bird per se. The marked crowing behavior of *hen 225* bore no graded relation to the amount of hormone administered or to the response to hormone as measured by comb growth. Apparently the innate masculine behavior pattern of crowing in this hen was strong or was activated to a high degree by the hormone. Noteworthy is the continuance of crowing behavior long after cessation of hormone treatment, this in a hen which was never observed to crow prior to treatment.

If the type of hormone administration was adequate for the induction of crowing behavior it would appear that female chicks, at least of the types used, gave a lesser response than male chicks. This contrast was quite marked not only in the frequency but also in the completeness of the action and in the length of time that this behavior persisted.

Noteworthy is the fact that masculinization of the female, be it morphological, physiological or psychological appears to be of the type characteristic of the male of that species. For example, masculinization of the female monkey produces a clitoris whose shaft and glans tend distinctly toward the type of penis seen in the male monkey (23). Androgenic stimulation of the prostatic lobes of the female rat causes secretion of fluid similar in appearance and in staining reaction (Bouin fixative, HE stain) to that observed in part of the prostate of the male. Analogous to the type of morphological response, hens and female chicks crow.

Evocation of patterns of masculine behavior in the female bird by administration of exogenous androgens, raises the question as to presence in other species of strongly masculine patterns of behavior that lie preformed but are at least partially latent until activated by male hormone substance. This recalls comments upon the masculine type of behavior exhibited by women with high amounts of male hormone as a result of adrenal or ovarian virilism or hermaphroditism. But it must be remembered that in the human being it is difficult to ascribe 'masculine' behavior to the influence of male hormone substances, for many other factors such as past experiences and the environment help to mould behavior and would tend to suppress, augment, modify or over-shadow hormonal influences. It seems certain that in lower animals, however, the absence of male hormone substance results in a lessening of 'masculine' activities in males and its presence is followed by a restoration of such activities in males and stimulation of such otherwise non-existent male-like activity in females. In eunuchoid and castrated men 'masculine' activity can be shown to be influenced by therapy with androgens, but the presence of any such influence in the female must be carefully and critically analyzed to avoid confusion with other factors. Especially pertinent is appreciation of the difficulties in recognition of what responses are completely or in some certain part 'masculine' behaviorisms and to distinguish these from attributes popularly thought of as being masculine.

Since the androgen-treated hens exhibited a mature type of crowing

without passing through an immature stage such as is seen in the week-old chick receiving androgens, the question may be raised as to what is responsible for the developmental changes in the syrinx of the growing pullet.

Both Deansley and Hill believe that ovarian grafts to the ears of castrated males do not produce testosterone, the only known (as yet) testicular hormone. It is worth noting, then, that a compound other than testosterone, the degradation product in human urine, androsterone, can produce behavioral responses like those of the cock.

These responses, in general, recall cock-like actions of chickens in which the right gonad began to function as testicle (40, 41), and should be regarded in relation to the larger problem of the capacity of the female for response to androgens and the influence upon this response of age, species, quantity and type of hormone and previous treatment.

SUMMARY

Administration of crystalline male hormone substances can produce definite cock-like behavior in either newly-hatched female chicks or in the mature hen. Following administration of testosterone propionate to the newly-hatched female chick the crowing response resembled that which can be produced in the male chick of the same age. The act of crowing involves complex body movements coordinated with vocalizations. The head is elevated, the neck arched and the neck feathers ruffled. These motions are accompanied at the proper time by opening of the bill and emission of a short high-pitched squeak.

The chicken not treated until 8 months of age and already laying eggs, may still react to testosterone propionate by crowing. The call is strident, lengthy and even when first observed is of a markedly mature nature without ever having been observed to pass through any immature stage such as is seen following androgen injections of newly-hatched chicks.

Androsterone was also capable of influencing crowing actions in a hen. These actions were similar to those occurring during administration of testosterone propionate.

The degree of crowing behavior was not proportional to the degree of comb growth, instead seeming to be largely dependent upon factors in the individual bird in addition to the degree of androgenic stimulation.

Potentially masculine behavior patterns appear to be preformed but latent in the female until activated by adequate stimulation by androgenic substances. They can be exhibited by the androgen-treated female even though no cock is present to serve as an example of such behavior. The type of masculine behavior shown by the female receiving male hormone substance tends toward the type of behavior shown by the male of the species. The limited data presented in this report would suggest, however, that at least under the experimental conditions employed, the females responded to the androgen, testosterone propionate, to a lesser degree than the males. Discussion is given of the ability of the female to produce and to respond to male hormone substances.

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THE COLCHICINE TEST AS A METHOD FOR DETERMINING THE TIME OF ONSET AND THE DURATION OF ACTION OF MALE ACTIVE SUBSTANCES

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THE ACTION of colchicine in arresting mitoses in the metaphase (1) has been used by several investigators for studying the growth processes brought about by hormones: by Allen, Gardner and Smith (2) for the action of estrogenic substances on the vagina and uterus of castrated mice, by Leblond and Allen (3) for the action of prolactin on the crop gland of pigeons and by Fleischmann and Kann (4) for the action of the thyrotropic hormone. Dustin and his school (5), Fleischmann and Kann (6), Manus (7) and Martins (8) have used colchicine independently of one another to formulate more clearly the test of Loewe and Voss (9), in which the action of male sex hormones on mitoses in the seminal vesicles of castrated mice was observed. As already recorded briefly (10) a combination of the two methods can be used for studying the onset of the action of male active substances.

MATERIAL AND METHOD

The experiments were carried out on 370 infantile, castrated, white, male mice, descended for the greater part from a pure bred strain produced by crossing first cousins for 20 generations. The animals were used for the experiment 12 to 14 days after castration. If the experiment is performed earlier (4 or 5 days after castration) the control animals, which have been treated only with colchicine, sometimes show mitoses and evaluation is more difficult. Each dose of male active substance was dissolved in 0.1 cc. olive oil and administered subcutaneously or, in some cases, intraperitoneally by a single injection. Various groups of animals were subsequently injected subcutaneously with colchicine after varying intervals of time. Unless otherwise stated the animals were killed with chloroform 11 hours after the colchicine injection, the seminal vesicles fixed while still at body temperature in Nawaschin's solution, embedded in paraffin, cut in series and stained with iron hematoxylin.

At first, in order to determine the latent period, 15γ of testosterone[1] or 18γ of testosterone propionate was used. In the course of the investigation, however, more equal distribution of the mitoses and a sharper delineation of the latent period were obtained by larger doses; 150γ of testosterone or 180γ of testosterone propionate was therefore used in all further experiments. Colchicine puriss. Merck was injected in aqueous solution (1 mg. in 10 cc. of distilled water) in doses of 0.5, 1 and 2γ per gm. of body weight. The latter amount, which represented the toxic limit for our

[1] The firm of Organon (Oss) generously supplied the testosterone and testosterone propionate used in these experiments.

TABLE 1

Male active substance and amount given	Colchicine[1] dose in γ per gm. body weight	No. of animals	Interval of time between injection of hormone and death in hours	No. of mitoses per field magnification 280×
Testosterone 150γ	½	5	10	½
	1	3	10	1
	2	5	10	4
	2	4	20	7
	½	5	23	1
	1	4	23	2
	2	5	24½	15
	2	5	26	14
	½	5	27	5
	1	4	27	18
	½	5	31	9
	½	6	34	12
	1	14	34	27
	½	2	40	20
	1	7	40	38
Testosterone propionate 180γ	½	5	10	0
	1	5	10	1
	2	4	10	2
	2	4	20	2
	½	3	23	1
	1	5	23	2
	2	4	24½	2
	2	4	26	2
	1	9	27	9
	2	4	27	15
	1	6	31	21
	½	3	34	20
	1	16	34	30
	½	2	40	27
	1	6	40	38
Controls with Colchicine only	½	15	—	1
	1	18	—	1½
	2	11	—	1½

[1] The animals were injected with colchicine 11 hours before death.

infantile mice was most suited for our purpose. All the sections were examined for mitoses, which were counted at a magnification of 280 diameters in the fields where they were most numerous. At least 20, usually more, fields were counted for each animal. The average number of mitoses per field was calculated for each animal and from these figures the mean value was determined for the entire group. Thus the mitoses numbers represent the average for areas with numerous mitoses for the whole animal group.

EXPERIMENTAL RESULTS

Observations on the duration of the latent period are given in table 1. From these it is seen that the latent period for testosterone is about 24 hours, while that for testosterone propionate is 27 to 31 hours. The activity begins somewhat sooner with testosterone, but is more uniform and the distribution of the mitoses more regular with the propionate, although with the latter an irregular distribution of the mitoses frequently occurs (5, 7).

In general, the distribution is more regular and uniform with larger doses, longer intervals after injection and the use of the propionate.

In order to determine whether or not the latent period is dependent solely on the rate of absorption from the site of injection, the hormone was

TABLE 2

Male active substance and amount given	Dissolved in 0.1 cc. of	Injection	Colchicine dose in γ per gm. body weight	No. of animals	Interval of time between injection of hormone and death in hours	No. of mitoses per field magnification 280×
Testosterone 150γ	oil	i.p.	2	4	27	28
	oil	s.c.	2	4	27	18.4
	oil	s.c.	2	3	11	2
	oil	i.p.	2	4	11	2
	propylene glycol	i.p.	2	5	11	6
	oil	s.c.	2	5[1]	5	2
	oil	i.p.	2	4[1]	5	4.7
	propylene glycol	i.p.	2	4[1]	5	3.8
Testosterone propionate 180γ	oil	i.p.	1	5[2]	24	13
	oil	s.c.	1	5[2]	24	6

[1] The animals were injected with colchicine 11½ hours before death.
[2] The animals were injected with colchicine 9 hours before death.

dissolved in propylene glycol and injected intraperitoneally in another group of animals. At autopsy, 5, 11 and 24 hours after injection, it was noted that in all the animals the injected substance was in direct contact with the seminal vesicles. In spite of the more rapid absorption from the peritoneal cavity and the presence of the solution on the surface of the seminal vesicle, the number of mitoses was not much increased (see table 2). It appears probable therefore that the duration of the latent period is not solely determined by the rate of absorption of the injected substance.

We have also used the colchicine test to investigate the action of 'X' substance mixed with testosterone as compared with testosterone and testosterone propionate alone.

X, which may be several substances, is found in the fatty acid fraction (unsaturated) and was first described along with testosterone (11). In spite of extensive investigations the substance has not yet been identified (12), but it is probably one of the higher unsaturated fatty acids. X mixed with free testosterone has the same intensifying effect as esterification with pro-

TABLE 3

Male active substance and amount given	Colchicine[1] dose in γ per gm. body weight	No. of animals	Interval of time between injection of hormone and death in hours	No. of mitoses per field magnification 280×
Testosterone 15γ	2	5	23	3
	2	5	33	8
	2	7	36	5.5
	2	7	40	4
Testosterone 15γ + substance X from 100 gm. of testis.	2	3	23	1
	2	3	33	4
	2	3	36	11
	2	3	40	16
Testosterone propionate 18γ	2	4	23	0
	2	5	33	11.5
	2	7	36	25.5
	2	7	40	30
Controls with colchicine only	2	6	—	1

[1] The animals were injected with colchicine 12 hours before death.

pionic or acetic acid and is therefore the pharmacological equivalent of these compounds. Like these compounds, X only intensifies those male active sterols possessing a hydroxyl group in position 17.

Manus (7) has demonstrated that X alone does not act as a male hormone and has no mitosis regulating properties but intensifies the action of male substances when injected with them.

We wished to establish whether X mixed with testosterone prolonged the latent period. It may be seen (table 3), that testosterone propionate produced more regular and more numerous mitoses than testosterone alone or testosterone mixed with X. However, when X was combined with testosterone the action was similar to that of testosterone propionate. The latent period was prolonged and an increase in and more uniform distribution of the mitoses were seen.

TABLE 4

Male active substance and amount given		Colchicine[1] dose in γ per gm. body weight	No. of animals	Interval of time between injection of hormone and death in hours	No. of mitoses per field magnification 280×
Testosterone	15γ	½	5	58	2.5
Testosterone propionate	18γ	½	5	58	11.0
Testosterone	150γ	½	4	58	14.0
Testosterone propionate	180γ	½	4	58	26.0
Controls with colchicine only.		½	3	—	0.3

[1] The animals were injected with colchicine 10 hours before death.

We have also tried to establish whether the colchicine test can be used for determining the duration of the activity of injected male substances. As is shown in table 4, the activity of 15γ of testosterone disappears after 58 hours (2 to 3 mitoses), while the equivalent amount of testosterone propionate, viz. 18γ, shows an average of 11 mitoses per field. When larger amounts are injected, activity persists for a longer time. Fourteen mitoses per field were seen 58 hours after injection of 150γ testosterone while 26 mitoses after 180γ testosterone propionate.

SUMMARY

Modification of the test of Loewe and Voss by the use of colchicine permits of a finer analysis of the effect of male hormones on the seminal vesicles than by the usual methods in which the enlargement of the whole seminal vesicle is observed. Observation of the action of a single injection of a male active substance throughout its course of action is made possible. On comparison of different active substances the more accurate histological analysis probably permits the establishment of differences in the mechanism of the action.

The first profuse formation of mitoses in the seminal vesicles of young castrated mice is observed 24 to 25 hours after injection of 150γ of testosterone and 27 to 31 hours after injection of 180γ of testosterone propionate. Their activity is still maintained after 58 hours, while doses ten times smaller develop much weaker activity after this time.

In general, testosterone propionate shows the most numerous mitoses and the most uniform distribution. The addition of 'X' substance to testosterone increases the activity of the latter.

I am greatly indebted to Miss M. Smit for the careful execution of the histological work, to Miss E. G. Simons and Mr. Witlam for much help during the work.

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THE URINARY EXCRETION OF ESTROGENS, ANDROGENS AND FSH FOLLOWING THE ADMINISTRATION OF TESTOSTERONE TO HUMAN FEMALE CASTRATES

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AMELIORATION of the vaso-motor symptoms of the menopause by the use of testosterone propionate has been reported by several investigators (1–4). Salmon (1) noted a change in the vaginal smear from the menopausal to the cornified type, which was not found by Shorr et al. (2). Both, however, observed a decrease in the levels of FSH after treatment with testosterone became effective. It was concluded that testosterone had the same effect as the estrogens, that is, inhibition of the anterior pituitary gland.

This study was undertaken to ascertain if there were changes from the pre-treatment levels of urinary FSH, androgens and estrogens, after administration of testosterone propionate[1] to female castrates. Similar work on normal males, eunuchoids and female castrates, to be referred to later in this paper, has been reported by others during the course of these experiments.

Subjects. Four women who had severe menopausal symptoms following surgical or radiation castration were selected for this study.

Patient No. 1, O. H., age 41, had a supravaginal hysterectomy and unilateral oöphorectomy 8 years before. Radiation therapy (1200 r) was given to the remaining ovary 2 years prior to the present observation.

Patient No. 2, F. B. P., age 35, had a supravaginal hysterectomy and bilateral salpingo-oöphorectomy 6 months before for fibroids of the uterus and cystic ovaries.

Patient No. 3, E. R., age 20, had a bilateral oöphorectomy 2 years previously for bilateral granulosal cell tumors.

Patient No. 4, M. L., age 30, had a radical mastectomy for carcinoma of the breast. X-ray therapy (1200 r) was given to the ovaries 1 year before this investigation.

Methods. Follicle stimulating hormone (FSH) was precipitated from the urine with ethyl alcohol (95%) and assayed on pure strain immature black female mice (Bar Harbor C57) by the technic of Zondek. The estrogens and androgens were extracted by the method of Smith and Smith (5). The extract was then divided into 2 equal portions and 1 portion was taken up in sesame oil and assayed for total estrogenic activity with a minor modification of the Allen-Doisy technic. The estrogens were separated from the androgens in the remaining half using a minor modification of the method of Gallagher et al. (6). The androgens were then titrated by

[1] We are indebted to Drs. Gregory Stragnell and Max Gilbert of the Schering Corporation and to Dr. Ernst Oppenheimer of the Ciba Company for a generous supply of testosterone propionate under the trade names of oreton and perandren, respectively.

the colorimetric method of Oesting (7). With this technic the non-phenolic androgens, some of which may be estrogenic and are insoluble in alkali remain in the neutral fraction, and are thus separated from the phenolic estrogens which are soluble in alkali.

Three complete 24-hour specimens of urine were obtained on all patients at intervals of several days before testosterone was administered. Testosterone propionate was then given, intramuscularly in the doses indicated, and assays of 24-hour specimens of urine for FSH, estrogens and androgens were done at the intervals indicated on the graphs. The results given for the estrogens represent the total activity of the

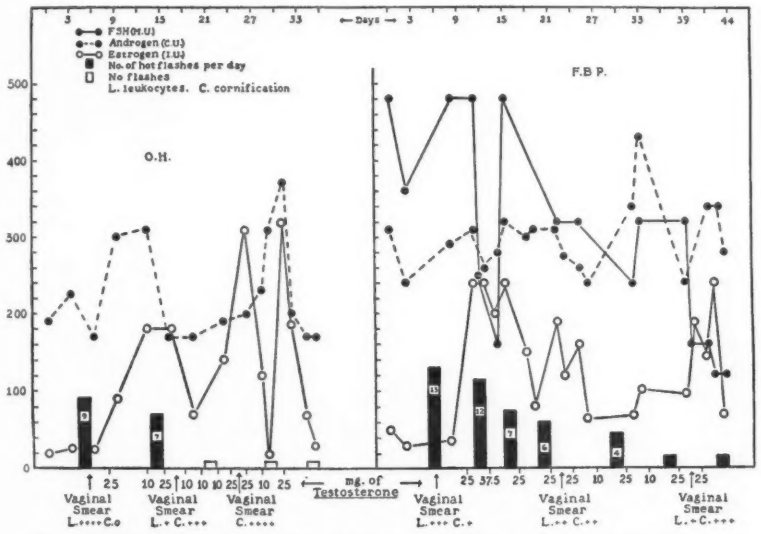

Fig. 1. M.U., Mouse Units; C.U., Color Units. One C.U. of urine extract is equivalent in biological activity to 10 γ of crystalline androsterone, while one C.U. of pure androsterone is represented by 90 γ of crystalline material. I.U., International Units, equivalent in biological activity to 0.1 γ of pure estrone.

specimen. We are now assaying the alkali-soluble or estrogenic fraction and the neutral or androgenic fractions for estrogenic activity. These will be reported later.

Results. From a clinical standpoint the following observations were made. Vaginal smears taken on the patient prior to treatment were of the menopausal type. All patients noted a definite and striking diminution in the number and severity of 'hot flashes' within 2 weeks after therapy was started. At about this time all noted a profuse vaginal discharge which contained many cornified cells. In 2 instances there was complete cornification with no leukocytes by the third week. In the other two cases, cornification was never complete though the vaginal smears showed a marked change from the pre-treatment appearance. All subjects noted an increase in libido without enlargement of the clitoris, growth of hair, or change in the voice. The graphs present the essential laboratory findings in this study.

FSH. It can be seen that there was a definite decrease in the *FSH* level which paralleled symptomatic relief. The testosterone was not given over a

sufficient period in this particular study to completely relieve the patients of their symptoms.

Androgens. There was not a consistent rise in the androgen levels after treatment, although at times a definite elevation was detected. No attempt was made to estimate the percentage of recovery in the urine after testosterone injections. In general, however, the rise in excretion checked fairly well with the findings of others (8, 9) and corresponded to the size of the dose.

Estrogens. There was a demonstrable rise in the total estrogenic activity

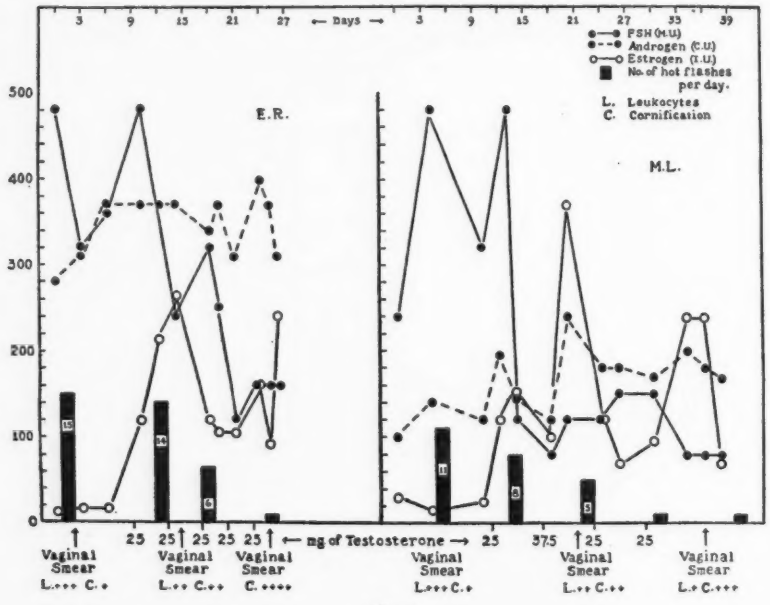

Fig. 2.

in every case. All assays yielded values moderately to markedly higher than the control levels. As in the case of the androgens the levels paralleled the amount of hormone injected. It can be seen that there was a distinct rise in the estrogen excretion even during the first 24 hours after the hormone was administered. This persisted for varying periods. The estrogens usually returned to the control level in 48 to 72 hours, following the last dose of the hormone.

DISCUSSION

These experiments indicate that there is an increase in the total urinary estrogen excretion in the human female castrate after administration of testosterone. This is contrary to the results reported by Laroche et al. (4) who found no change in the urinary estrogen excretion after the injection of testosterone to similar subjects. Their method of extraction and assay of the estrogens is not given.

Kochakian (11) could not find an increase in estrogen excretion after administration of testosterone to castrated male dogs. This was based on a separation of the neutral from the phenolic fraction. Recently Hoskins et al. (12) reported an increase in both androgens and estrogens after administration of testosterone to human male eunuchoids. This was performed on extracts in which the androgen fraction was separated from the phenolic fraction. They found very slight estrogenic activity in the non-phenolic or neutral fraction and no androgenic activity in the phenolic fraction. Steinach and Kun (13) have reported an increase in the estrogen excretion after the administration of testosterone and androsterone to normal human males. The values for estrogens were considerably higher than those published by the above investigators. This work, however, was done on normal males and no attempt was made to separate the androgenic and estrogenic fractions. The latter two groups of investigators believe the increase in estrogens is due to a conversion from the androgens. Further evidence for this is found in the stallion which excretes excessive amounts of estrogen, which Zondek (14) interprets as a conversion. The mechanism for this is not clear, but conversion into estradiol and its degradation products, although obviously complicated remains a possibility.

The recovery of estrogens and androgens even in individuals in whom the gonads are removed indicates, as others have suggested, an extra-gonadal source of these substances.

The rise in estrogens may well account for the increased cornification seen in the vaginal smears. It is interesting in this regard that testosterone, although it is not estrogenic in the mature spayed rat, will produce vaginal cornification in the intact immature rat (15, 16), as well as in the ovariectomized (16, 17) and hypophysectomized immature rat (16).

It is surprising that the injection of androgens does not result in a greater increase in the androgen excretion. It suggests that the hormone may be (*a*) converted to a true estrogen, (*b*) an androgen with estrogenic activity, (*c*) metabolized to an inactive form, (*d*) destroyed.

These possibilities are based on the findings of the investigators mentioned above, as well as those of our own. The indicator (m-dinitrobenzene) used in the colorimetric method for the androgens may combine with biologically inert androgens thus yielding values which are higher than those obtained by bioassay. It seems significant, therefore, that a marked rise in androgens cannot be demonstrated even by this method. Further information may be forthcoming when our assays of the neutral and alkali phenolic fractions are completed.

The effect on the FSH levels is in accord with the other investigators (1, 2, 4). This suggests that androgens exert an inhibitory action upon the secretion of the anterior pituitary gland, an effect which is similar to that exerted by the estrogens.

SUMMARY

Testosterone propionate when administered to human female castrates results in (*a*) a discernible increase in the urinary total estrogen levels, (*b*) a slight rise in the urinary androgen excretion, (*c*) a decrease in the urinary

FSH levels, (*d*) a cornified vaginal smear and (*e*) symptomatic relief from the vaso-motor symptoms of the menopause. It appears, therefore, that there is a distinct correlation between the laboratory findings and the clinical changes.

We are indebted to Dr. Joseph C. Aub for valuable suggestions in the preparation of this paper.

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THE INFLUENCE OF ANTERIOR PITUITARY EXTRACTS ON THE PROTEINS OF THE LIVER[1]

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LEE (1) was first to definitely demonstrate the relation between the anterior pituitary growth hormone and protein metabolism. That a hormone influencing protein metabolism was *an* if not *the* active principle of the growth hormone was shown by an increased percentage protein nitrogen content of the body as compared with a pair-fed litter-mate rat. Hence, it was suggested that the unique action of the anterior pituitary in stimulating body growth is due to a growth hormone which acts as an essential part of the mechanism, controlling synthesis, deposition and utilization of protein in the tissue.

Nitrogen retention was indicated upon injection of AP growth hormone extracts in dogs, rabbits and rats by Teel and Watkins (2), Teel and Cushing (3), and Lee and Shaffer (4) who observed a lowering of blood nitrogens and urinary nitrogen. Hypophysectomized rats were found (5) to have a reversed picture, that of an increased nitrogen excretion. It has been shown that hypophysectomized rats have lowered basal calorie metabolism and endogenous protein metabolism and storage protein over their normal controls (6).

The livers of animals have been shown to be affected by pituitary extracts. Shaffer and Lee (7) observed upon injection of AP growth extracts a decrease of free amino acids, peptides and urea in the liver, while the ammonia increased. A slight hypertrophy of the liver was found. Hypophysectomy caused a reverse condition (8).

Using a histological technic, Liang and Wu (9) described a substance found in the blood of fasting dogs and cats which when injected into rats caused the disappearance of the protein reserve of the liver. A substance identical with that found in the blood was extracted from the anterior pituitary. The substance causing this effect was found not to be identical with any other recognized pituitary hormone nor with insulin, thyroxine or adrenal hormones.

While we were investigating the fat metabolism hormone (10), a change in the appearance of the livers was noted. We became interested in determining whether or not the chemical composition, other than the fatty infiltration, had been changed by the AP extract. The protein partition was thought of as a possible avenue of change. If the extract influenced the proteins of the liver, the conclusion would be reached that there was a protein metabolism hormone present in the pituitary.

The object of the present paper is to present the results of the study of the influence of AP extracts upon the proteins of guinea pig livers.

[1] The data presented in this paper were submitted to the Graduate School, University of Missouri in partial fulfilment of the degree Master of Arts, 1939. Grateful acknowledgments are made to Professors Addison Gulick, C. W. Turner, L. E. Thomas, and to Mr. A. J. Bergman for valuable assistance.

EXPERIMENTAL

Methods. Immature female guinea pigs obtained from a commercial breeder were kept for several days in a temperature regulated room. Food of good quality was kept before them at all times. In case the animal was not healthy in appearance and gaining weight before the start of the experiment, it was not used.

Pituitary extracts were administered as explained later. Immediately after killing and bleeding, the livers were removed, freed of foreign tissue and external blood, and weighed. They were then diced and the fat extracted with hot alcohol and ether mixture (3:1). After extraction and drying, the defatted, dehydrated liver was weighed and ground for protein extraction.

The fractionation of the liver proteins presented some difficulty since it was necessary to precede it with a fat extraction in studying the fat metabolism hormone. The water-soluble and salt-soluble proteins of former investigators (11) became denatured at this stage and added themselves to the insoluble residuary fraction. Since the alkali-soluble protein apparently was not disturbed, this protein was selected for study. Extraction was carried out by a modification of the method described by Luck (11) and his associates (12, 13). With the use of potassium iodate as an inhibitor of proteolytic enzymes of the liver (14), the extraction was made with .25% NaOH at room temperature. The alkali-soluble protein was precipitated by adjusting the reaction to between pH 5 and pH 5.5 and allowing to stand over night at a cold temperature. The residue was washed with 1% acetic acid. Both products were centrifuged and dried with alcohol and ether. The protein was determined gravimetrically. Arginine and tryptophane determinations on the proteins were made by the method described by Thomas et al. (15) and Thomas (16).

The AP extracts used in the experiment here reported consisted in the initial extract of cattle pituitaries prepared by Bergman and Turner (17). This extract is known to be rich in lactogenic, carbohydrate metabolism, fat metabolism, gonadotropic, thyrotropic and possibly other hormones. The initial extract has been further separated by Bergman and Turner (18) into lactogenic and thyrotropic fractions. The former has been found to be quite free of the other hormones whereas the latter contains the thyrotropic, carbohydrate metabolism and gonadotropic hormones as well as very small amounts of the fat metabolism hormone (19). For purposes of injection, the dried preparation was dissolved in slightly alkaline water, then precipitated by bringing to the isoelectric point with HCl. Injections of this suspension were made at different times during the day, usually in the morning, and the animal killed at varying intervals up to a maximum of 26 hours after injection. Controls were killed at times during the day and night to correspond with the time that the injected animals might be killed.

Observations. No unequivocal increase was found in the moist weight of the livers of the experimental animals over the weight of the controls (table 1). When the longer intervals after injection of 20 mg. of APE were taken, the livers showed some slight increase of weight. These 3 groups that

had proportionately larger livers than the normal group comprised too few guinea pigs to be statistically significant. The injection of 35 animals with amounts of the extract ranging from 1 to 10 mg. (not included in table 1) caused no hypertrophy but the values varied somewhat. Since the 3 groups were killed after longer periods of treatment, one might imagine a trend toward a greater hypertrophy as the period of time increases. However, it was observed that the groups injected with 10 mg. of the APE, the animals being killed at 24 hours, did not show this seeming hypertrophy. Likewise, several other extracts did not affect the liver weight.

The weight of the dehydrated, defatted liver was found to vary only slightly. Analysis of the data shows that the variation could not be counted

TABLE 1. THE RELATION OF LIVER COMPONENTS TO BODY WEIGHT AND THE WATER CONTENT OF GUINEA PIG LIVERS AFTER INJECTION OF AP EXTRACTS, INDETERMINATE EFFECT

Treatment	Interval of time	No. animals	Body wt.	Liver		Dried Liver		Water content	Dry wt.
				Total wt.	100 gm. body wt.	Total wt.	100 gm. body wt.		
	hr.		*gm.*	*gm.*	*gm.*	*%*	*gm.*	*%*	*%*
Normals		24	186.5	6.6007	3.5380	1.8013	1.011	66.52	29.0
10 mg. APE[1]	6	43	182.1	6.7846	3.7258	1.8708	1.034	66.78	26.7
10 mg. APE[1]	24	8	156.8	6.0296	3.8454	1.6332	1.027	66.73	26.6
20 mg. APE[1]	6	9	179.1	7.0145	3.3165	2.0265	1.073	64.44	28.7
20 mg. APE[1]	8	8	186.9	7.4261	3.9733	1.8637	.997	65.74	25.1
20 mg. APE[1]	12	5	163.0	7.3455	4.5064	1.6447	1.045	67.14	22.9
20 mg. APE[1]	24	6	157.0	7.1061	4.5262	1.7519	1.126	68.14	24.7
20 mg. APE[1]	26	3	184.3	9.0499	4.9104	2.7129	1.484	—	29.9
100 mg. AD[2]	6	6	175.9	6.2987	3.5808	1.5682	.891	69.75	24.9
12 mg. APE[3]	6	6	174.5	5.8733	3.3668	1.4304	.820	68.25	24.4
10 mg. APE[4]	6	6	182.8	7.0574	3.8620	1.6670	.919	70.23	23.6
100 mg. CL[5]	6	7	187.4	6.5792	3.5120	1.6103	.962	70.07	24.5
75 mg. CTF[6]	6	9	190.7	6.7001	3.5140	1.5804	1.815	69.32	23.0

[1] Initial extract of AP.
[2] Cattle acetone-dried AP.
[3] Initial extract of AP prepared fall 1938.
[4] Sheep initial extract of AP prepared 1936.
[5] Cattle-lactogenic fraction from initial extract used in tables 1, 2, 3.
[6] Cattle-thyrotropic fraction from initial extract used in tables 1, 2, 3.

as definitely significant, as the number of animals in the group was small. Also the fat-free dry weight of the livers represented a fairly constant percentage of the total body weight. The variations observed did not stand in any definite relation to the amount of hormone injected or to the interval before killing. Even though the fat content of the livers was observed to increase after hormone injection, the water percentage was not altered to any great extent. Thus, judging superficially, the conclusion might be reached that the livers were not altered extensively by the hormone treatment.

Although it has been shown that the initial extract of the anterior pituitary does not produce a true hypertrophy, the likelihood should be kept in mind that the hormone extract may nevertheless have some influence on the livers of the animals. All considerations of protein metabolism show that

the liver functions very actively in all phases of protein anabolism or catabolism. If one supposes growth to occur through a synergism of several pituitary hormones, a protein metabolism hormone must then needs be present that would induce greater activity of the protein metabolism of the liver.

Our data showed that the livers of the normal animals contained on the average 36.75% alkali-soluble protein (table 2). This value as percentage of total liver (10.0% of moist weight) was somewhat higher than the value Luck (11) gave for the alkali-soluble protein of the rat liver. Several animals injected with casein and liver extract were included as normals.

If animals were injected with 20 mg. of the initial extract and then killed at varying intervals from 4 to 26 hours, there resulted a definite change of the liver protein picture (table 2). The alkali-soluble protein showed a large decrease. This decrease was as great as 53% of the normal level in one instance. A progressive decrease was noted up to a 12-hour interval with a fluctuation at longer intervals. There was no overlapping of the range of values of the residue protein (including the denatured fraction). Although it

TABLE 2. A STUDY OF THE ALTERATION OF THE PROTEINS OF THE LIVER INDUCED BY INJECTION OF THE AP EXTRACT (20 MG. APE INJECTED)

No. animals	Interval of time	Dried liver wt.	Alkali-sol. protein			Residue protein	Soluble material	Total protein
			%					
			ave.	range				
				high	low			
	hr.	*gm.*				%	%	%
27	Normals	1.8013	36.75	41.99	32.57	43.49	19.76	80.24
4	4	1.6785	26.83	30.73	22.77	51.73	21.44	78.56
9	6	2.0265	24.59	30.09	16.11	51.54	23.87	76.13
8	8	1.8637	24.05	28.90	17.22	47.65	28.30	71.70
5	12	1.6447	17.35	24.24	13.00	60.39	22.26	77.74
6	24	1.7519	22.48	30.20	19.91	48.53	28.99	71.01
3	26	2.7129	17.22	23.46	8.27	55.86	26.92	73.08
	10 mg. APE							
8	4	2.2320	25.76	33.50	20.76	50.28	23.44	76.56
8	24	1.6332	22.10	25.49	19.63	50.28	27.62	72.38

showed considerable variation, it was invariably higher in the livers of the injected animals than in the livers of the normals. The total of the soluble materials (such as glycogen, glucose, amino acids, creatine, creatinine and urea) was increased slightly. Total protein decreased.

The injection of varying dosages of the initial extract resulted in decreases of the alkali-soluble protein at the end of 6 hours equivalent to the change observed in the preceding group (table 3). The 5 mg. level caused as great a decrease of this fraction as that of any other level. Contrary to what one might expect at first, increasing levels of hormone injection at this interval of time was found not to yield a further decrease of the alkali-soluble protein. These data would suggest that very small amounts of the extract were adequate to produce the full effect.

TABLE 3. A STUDY OF THE ALTERATION OF THE PROTEINS OF THE LIVER INDUCED BY INJECTION OF VARYING AMOUNTS OF THE AP EXTRACT (KILLED AT 6 HOURS)

No. animals	Amounts APE injected	Dried liver wt.	Alkali-sol. protein			Residue protein	Soluble material	Total protein
			%					
			ave.	range				
				high	low			
		gm.				%	%	%
27	Normals	1.8013	36.75	41.99	32.57	43.49	19.76	80.24
8	5 mg.	1.9477	19.51	23.31	14.96	46.28	34.21	65.79
8	8 mg.	1.6751	23.51	32.53	22.43	54.44	22.05	77.95
27	10 mg.	1.8176	22.12	28.83	14.92	52.33	22.55	74.45
7	12½ mg.	1.8335	22.74	26.83	20.18	50.16	27.10	72.90
4	15 mg.	2.0891	24.81	28.54	14.56	48.93	26.24	73.74
9	20 mg.	2.0265	24.59	30.09	16.11	51.54	23.87	76.13

The acetone-dried preparation of a different batch of cattle pituitaries caused slight change of the liver proteins (table 4). Aqueous extracts from these pituitaries effected a change in the proteins in proportion to the alteration caused by equivalent amounts of an acetone-dried preparation. An extract of sheep pituitaries seemed to be only slightly active.

Fractions of the APE were observed to have very slight efficacy (table 4). The lactogenic fraction at 100 mg. levels had only a small amount of the active principle present. The thyrotropic fraction had medium activity in 75 mg. but was very poor in producing a liver protein change.

Some thought should be devoted to the question whether this physiological-chemical reaction is parallel with the fatty infiltration of the liver due to the fat metabolism hormone. Liang and Wu (9) found by their observations that their protein metabolism hormone could not be identical with the fat metabolism hormone due to its difference in physical properties. Fur-

TABLE 4. THE EFFECT OF VARIOUS AP EXTRACTS AND EXTRACTIVE FRACTIONS ON THE LIVER PROTEINS OF GUINEA PIGS (KILLED AFTER 6 HOURS)

Treatment	No. animals	Dried liver wt.	Alkali-sol. protein	Residue protein	Soluble fraction	Total protein
		gm.	%	%	%	%
50 mg. AD[1]	5	1.4419	33.76	37.04	29.20	70.80
75 mg. AD[1]	6	1.6434	30.52	38.09	31.39	68.61
5 mg. APE[2]	6	1.5150	32.76	33.74	33.50	66.50
10 mg. APE	6	1.6485	25.85	53.55	20.60	79.40
12 mg. APE[2]	6	1.4304	30.58	49.61	19.81	80.19
10 mg. APE[3]	6	1.6770	37.70	32.84	29.46	70.54
100 mg. CL[4]	6	1.6103	34.41	38.49	27.10	72.90
75 mg. TF[5]	5	1.6291	28.67	36.09	35.24	64.76
10 mg. xx[6]	4	1.6329	32.09	34.06	33.85	66.15

[1] Cattle acetone-dried AP.
[2] Initial extract of AP prepared fall 1938.
[3] Sheep initial extract of AP prepared 1936.
[4] Cattle-lactogenic fraction from initial extract used in tables 1, 2, 3.
[5] Cattle-thyrotropic fraction from initial extract used in tables 1, 2, 3.
[6] A discarded powder from fractionation of other hormones.

TABLE 5. LOSS OF EFFECT THROUGH TREATING THE APE WITH .1 N NaOH AND HEATED TO 60°C. FOR 90 MINUTES. (10 MG. INJECTED, KILLED AFTER 6 HOURS)

Number	Body wt.	Blood fat	Liver fat	Dried Liver wt.	Alkali-sol. protein	Residue protein	Soluble non-protein fraction
	gm.	mg. %	%	gm.	%	%	%
Ave. Normals	180.5	60.4	4.68	1.8013	36.75	43.49	19.76
Ave. Control[1]	182.1	29.1	7.20	1.8176	22.12	52.33	25.55
1	179.0	25.0	4.04	1.7690	43.35	40.48	16.17
2	160.5	14.0	5.20	1.6699	34.76	34.76	28.07
3	184.0	10.0	6.50	2.0011	36.61	41.11	22.28
4	170.0	18.0	7.67	1.9807	36.61	38.59	24.80
5	185.0	25.0	8.64	2.1935	40.99	41.06	17.95
6	179.0	29.0	7.41	1.7190	38.17	39.99	21.84
7	167.5	33.0	7.92	1.8918	38.42	39.74	21.84
8	190.0	30.0	8.11	2.1091	41.00	38.76	20.24
Ave.	176.9	23.0	6.94	1.9188	38.71	39.61	21.65

[1] Injected with untreated APE.

ther they found it to be labile to heat if applied long enough and also to .1 N alkali or acid. For the purpose of studying lability of the hormone, we dissolved the initial extract in .1 N NaOH as a 1% solution and heated it for 90 minutes at 60° C. This treated hormone was injected at 10 mg. level and the animals were killed after 6 hours (the optimal time for the fat metabolism hormone action, 10). It was shown that the protein metabolism hormone had been destroyed (table 5). Assay of this preparation showed that its fat metabolism activity remained unimpaired. Therefore, it seems conclusive that this active principle in protein metabolism and the fat metabolism hormone are not identical.

To confirm that the protein extracted from the livers by the alkaline solu-

TABLE 6. QUANTITATIVE ANALYSIS OF THE ALKALI-SOLUBLE PROTEIN EXTRACTED FROM LIVERS OF NORMAL AND TREATED GUINEA PIGS

Exp. No.	Treatment	Interval of time	Arginine	Tryptophane
		hr.	%	%
1	Control		5.30	1.40
2	Control		5.30	1.38
3	Control		5.35	1.38
4	Control		5.32	1.38
5	10 mg. APE	6	5.30	1.40
6	10 mg. APE	6	5.30	1.39
7	15 mg. APE	6	5.30	1.37
8	20 mg. APE	6	5.30	1.39
9	20 mg. APE	8	5.40	1.38
10	20 mg. APE	24	5.35	1.38
11	50 mg. AD	6	5.40	1.36
12	100 mg. CL	6	5.35	1.32

tion was the same in the treated as in the normals, and to study further the residue proteins, determinations of arginine and tryptophane were made on a number of samples chosen at random. It was observed that the arginine and tryptophane of all alkali-soluble protein samples investigated were virtually the same and well within experimental error for the determination (table 6). It was concluded that without a doubt this protein was identical in all livers examined from both injected and normal animals. The arginine and tryptophane of the residue protein in the livers of the treated animals was considerably different from the control figures and showed considerable scatter (table 7). The trend of the variation was toward an increase in arginine content in the residual plus denatured protein from the treated animals over that of the normals. The tryptophane content showed a similar tendency but not as definite as that of the arginine.

TABLE 7. QUANTITATIVE ANALYSIS OF THE RESIDUE PROTEIN OF LIVERS FROM NORMAL AND TREATED GUINEA PIGS

Exp. No.	Treatment	Interval of time	Arginine	Tryptophane
		hr.	%	%
1	Control		3.60	1.55
2	Control		3.60	1.56
3	Control		3.80	1.70
4	Control		3.60	1.59
5	15 mg. APE	6	3.68	1.60
6	20 mg. APE	4	4.00	1.95
7	20 mg. APE	6	4.12	2.00
8	20 mg. APE	12	3.30	1.56
9	20 mg. APE	12	6.88	1.71
10	20 mg. APE	26	5.12	2.09
11	20 mg. APE	26	3.68	1.45
12	50 mg. AD	6	4.91	1.82

DISCUSSION

Attention was first drawn to protein metabolism as affected by AP hormones as a result of the study of growth hormones. More recently, the belief is being held that the growth hormone is a synergism of the other pituitary hormones combined. The protein metabolism hormone, then, has been thought of as the limiting factor since protein anabolism seems to be the most active phase and most significant as a stimulator of growth.

What the relation of the growth hormone to the protein metabolism hormone may be, has not been definitely determined. They may be identical. However, it has been suggested (20, 21) that AP extracts exert a stimulative action through the adrenotropic hormone and a decrease of catabolism through the growth hormone. Mirsky and Swadesh (22) suggest that the action may be partially through the pancreas. The data of Liang and Wu (9) have somewhat discredited these observations. They describe a protein metabolism hormone as being an entity separate from all other pituitary hormones, as well as from insulin and adrenal cortical hormones.

The data presented in this paper clearly indicate that the protein of the liver is altered by the AP extract used. Surprisingly enough, the effect is a decrease of the protein fraction that is considered to have the largest molecule. These findings suggest the presence of a protein metabolism hormone in the anterior pituitary, that has a direct stimulation on the liver and its manipulation of protein.

The biochemical mechanisms responsible for these changes can only be speculated on. It seems plausible that some enzyme system of the liver may be stimulated to change the alkali-soluble protein into one or several of the other proteins and finally into nitrogenous products required for protein anabolism in the body. Or the plasma protein might be raised due to an influx of this globulin into the blood stream (23). Possibly both mechanisms are at work. Further study of the liver and blood protein fractions will be necessary to throw light on the mechanism involved.

These data presented suggest that the decrease of the alkali-soluble protein of the liver could be profitably developed as an assay technic for this protein metabolism hormone. Small amounts of the hormone are shown to be effective enough to make the assay sensitive. Such an assay would be specific for the protein metabolism hormone.

SUMMARY

The injection of an initial extract of the anterior lobe of cattle pituitaries at levels from 1 to 20 mg. did not cause a true hypertrophy of the entire liver. The dehydrated, defatted weight of the liver per 100 gm. body weight was observed to be uniformly similar to the normals. The percentage of water in the liver did not vary significantly.

The alkali-soluble protein of the liver was decreased by a principle present in the anterior pituitary, denoted by the term protein metabolism hormone. This decrease of the protein fraction was 35 to 53% of the normal. An increase was noted in the protein fraction containing insoluble proteins and proteins that were originally soluble in water or dilute salts; also an increase of the soluble non-protein materials. Different levels of the hormone injection from 5 mg. to 20 mg. gave like responses, the indication being that the optimal level is low. The interval of time before killing was shown to affect the response slightly.

Acetone-dried preparations of a recently acquired batch of cattle pituitaries were observed to have a slight activity. Extracts of these pituitaries, in amounts corresponding to the acetone-dried preparations used, showed activity similar to the degree of activity produced by the acetone-dried powders.

Fractions of the initial extract rich in the lactogenic hormone and in the thyrotropic, carbohydrate metabolism and gonadotropic hormones, were found to contain little or no protein metabolism hormone. The protein metabolism hormone was further shown to be an entity by its being inactivated by .1 N NaOH at 60° C. for 90 minutes, while the potency of the fat metabolism hormone remained constant.

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THE EFFECT OF PITUITARY EXTRACTS ON KETONURIA, FAT CONTENT AND FAT DISTRIBUTION IN THE LIVER AND TISSUES OF MICE

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KETOGENIC activity of pituitary preparations was first demonstrated by Burn and Ling (1). Further investigations have shown that heat treatment in an alkaline medium inactivates such preparations, and Shipley and Long have reported on the complete inactivation of ketogenic potency following heat treatment (2, 3). We recently pointed out (4) that, in a previous communication (5), in which it was shown that ketonemia could be produced by certain extracts which had been heat treated in an aqueous alkaline medium, we had not sufficiently stressed the fact that by far the major part of ketogenic activity present in the original extract had been removed by the fractionation procedure. In the experiments which are now reported it is shown that the major part of the ketogenic activity in anterior lobe extracts is present in the protein fractions, and that in the case of most of these boiling in dilute acid medium does not remove the ketogenic property. The effect of various extracts of pituitary tissue upon the neutral fat and fatty acid content of the liver and tissues of mice are also included in this communication.

MATERIALS AND METHODS

Acetone bodies. In most of our earlier work on this subject we have used ketonuria and ketonemia of treated fasting rats as an index of the ketogenic potency of the extracts tested. Recently we have found that for such studies pure strain mice (dba) are the most satisfactory test objects. Male or female animals, 4 weeks of age, have been used. They were kept in specially constructed metabolism cages of a modified Hopkins type (4 in each cage). The urine was collected separately from the feces and preserved in copper sulphate solution. Total acetone bodies were determined by the gravimetric method of Van Slyke (6). The animals were fasted for the duration of the experiment (46 hours). Injections were made subcutaneously in 4 equal fractions at 2, 6, 21 and 30 hours respectively after the beginning of the fast. The total dosage is indicated in terms of gram equivalents of fresh tissue and the results are expressed as mg. total acetone bodies in terms of acetone per 100 gm. mouse in 46 hours (table 1).

Neutral fat and fatty acids. The fraction soluble in both hot ether-alcohol (1:4) and cold chloroform is referred to as the neutral fat fraction. Fatty

TABLE 1. THE EFFECT OF PITUITARY PREPARATIONS ON THE LIVER FAT AND KETONURIA IN MICE (dba) (4 mice in each test)

Extract	Dose gm.: original tissue	Sex	Acetone bodies excretion mg. acetone: 100 gm. mouse 46 hours	Liver neutral fat gm.: 100 gm. mouse	Liver fatty acids gm.: 100 gm. mouse
Control: Saline	0.5 c.c.	M	1.53	0.45	0.12
Control: Saline	1.0	F	2.06	0.45	0.15
Control: Muscle	0.2	M	2.50	0.52	0.11
Control: Muscle	0.4	F	2.13	0.50	0.15
A 1	0.08	M	2.53	0.53	0.15
	0.2	M	8.77	0.57	0.16
	0.4	M	28.5	1.08	0.18
A 2	0.08	M	1.23	0.43	0.12
	0.2	M	8.40	0.50	0.13
	0.4	M	14.1	0.61	0.19
A 3	0.08	F	5.62	0.60	0.14
	0.16	F	9.32	0.59	0.15
	0.2	M	32.8	0.71	0.21
	0.4	M	61.9	1.22	0.20
A 4	0.2	M	2.37	0.58	0.16
	0.4	M	2.37	0.43	0.13
A 5	0.2	M	6.50	0.61	0.12
	0.4	M	15.2	0.71	0.15
A 6	0.2	F	2.01	0.50	0.11
	0.4	M	1.37	0.41	0.14
B 1	0.08	M	4.61	0.39	0.13
	0.2	M	8.62	0.61	0.12
	0.4	F	28.1	0.77	0.18
B 2	0.08	F	3.90	0.43	0.14
	0.16	F	6.01	0.49	0.13
	0.2	F	7.22	0.60	0.12
	0.32	F	27.5	—	—
	0.4	F	36.4	0.97	0.21
B 3	0.2	F	23.8	—	—
	0.4	F	40.6	0.98	0.20
B 4	0.2	M	3.41	0.43	0.13
	0.4	F	1.72	0.51	0.12
B 5	0.2	F	2.20	0.49	0.14
	0.4	F	2.55	0.53	0.13
B 6	0.2	F	5.50	0.54	0.14
	0.4	M	21.8	—	—

acids were determined on an aliquot of the neutral fat fraction by the saponification procedure (7). The results are expressed as gm. fatty acids in terms of palmitic acid per 100 gm. mouse (tables 2 and 3).

Four weeks' old dba mice were also used to determine the effect of the extracts upon neutral fat and fatty acid content of the whole animal and also of the liver and tissues. The animals were fasted for 24 hours, unless otherwise stated, and the injections were made subcutaneously in 3 equal fractions at 1, 5 and 19 hours respectively after the beginning of the fast. The animals were anesthetized with amytal, and frozen with carbon dioxide ice prior to the determination of total body fat. When liver and other tissue fat were determined separately, the tissue was thoroughly ground in sand before extraction. The method of extraction and fatty acid determination was the same as that given above. The amount of extract injected at any one time was as a rule 0.25 or 0.5 cc.

Figure 1 gives a diagrammatic representation of the method of preparation of most of the pituitary extracts used. The control muscle preparation was prepared similarly to that of fraction A3 in figure 1. Preparation 622 (Jan. 17) was prepared from whole glands (5). Extract H4 was prepared from

TABLE 2. THE EFFECT OF PITUITARY PREPARATIONS ON BODY FATTY ACIDS OF MICE (dba) (Fasted 24 hours)

	Sex	Fatty acids per 100 gm. mouse	Fatty acids per 100 gm. mouse		% fatty acids distribution	
			Liver	Remainder of mouse	Liver	Remainder of mouse
		gm.	gm.	gm.		
Saline: 0.75 cc. in 24 hr.	F	1.25	0.15	1.10	11.9	88.1
Saline: 0.75 cc. in 24 hr.	F	1.61	0.17	1.44	10.5	89.5
Saline: 0.75 cc. in 24 hr.	F	1.26	0.16	1.10	12.8	87.2
Saline: 1.5 cc. in 24 hr.	M	1.25	0.14	1.11	11.6	88.4
Saline: 1.5 cc. in 24 hr.	M	1.70	0.17	1.53	9.8	90.2
Saline: 1.5 cc. in 24 hr.	M	1.12	0.14	0.98	12.2	87.8
Muscle: 0.3 gm. in 24 hr.	F	1.25	0.15	1.10	11.9	88.1
Muscle: 0.3 gm. in 24 hr.	F	1.51	0.17	1.31	11.5	88.5
Muscle: 0.3 gm. in 24 hr.	F	1.26	0.14	1.12	10.8	89.2
A 3: 0.15 gm. in 24 hr.	F	1.43	0.54	0.89	37.8	62.2
A 3: 0.15 gm. in 24 hr.	F	1.37	0.33	1.04	24.2	75.8
A 3: 0.15 gm. in 24 hr.	F	1.18	0.19	0.99	16.2	83.8
A 3: 0.3 gm. in 24 hr.	F	1.17	0.22	0.95	19.1	80.9
A 3: 0.3 gm. in 24 hr.	F	1.28	0.24	1.04	19.1	80.9
A 3: 0.3 gm. in 24 hr.	F	1.25	0.24	1.01	19.6	80.4
B 3: 0.15 gm. in 24 hr.	F	1.01	0.38	0.63	17.3	82.7
B 3: 0.15 gm. in 24 hr.	F	1.39	0.24	1.15	17.0	83.0
B 3: 0.15 gm. in 24 hr.	F	1.03	0.17	0.86	16.3	83.7
B 3: 0.3 gm. in 24 hr.	F	1.26	0.13	1.13	10.7	89.3
B 3: 0.3 gm. in 24 hr.	F	1.52	0.32	1.20	21.0	79.0
B 3: 0.3 gm. in 24 hr.	F	1.80	0.40	1.40	22.4	77.6
H 4: 0.15 gm. in 24 hr.	F	1.05	0.07	0.98	6.8	93.2
H 4: 0.15 gm. in 24 hr.	F	1.04	0.08	0.96	7.5	92.5
H 4: 0.15 gm. in 24 hr.	F	0.86	0.07	0.79	8.3	91.7
H 4: 0.15 gm. in 24 hr.	F	0.92	0.07	0.85	7.6	92.4
H 4: 0.15 gm. in 24 hr.	F	1.09	0.09	1.00	8.1	91.9
H 4: 0.15 gm. in 24 hr.	F	0.88	0.09	0.79	10.3	89.7
622 (Jan. 17): 1.0 gm. in 24 hr.	F	0.88	0.05	0.83	5.4	94.6
622 (Jan. 17): 1.0 gm. in 24 hr.	F	1.02	0.05	0.97	5.1	94.9
622 (Jan. 17): 1.0 gm. in 24 hr.	F	0.96	0.09	0.87	9.4	90.6
622 (Jan. 17): 1.0 gm. in 24 hr.	F	0.815	0.07	0.745	8.5	91.5
622 (Jan. 17): 1.0 gm. in 24 hr.	F	0.725	0.06	0.665	8.3	91.7
622 (Jan. 17): 0.4 gm. in 24 hr.	F	0.84	0.085	0.755	10.0	90.0
622 (Jan. 17): 0.4 gm. in 24 hr.	F	0.845	0.085	0.76	10.0	90.0
622 (Jan. 17): 0.4 gm. in 24 hr.	F	0.86	0.06	0.80	6.9	93.1

dissected posterior lobes of beef pituitaries and represents a fraction described (8) in another communication.

RESULTS AND DISCUSSION

The effect of pituitary preparations on ketonuria and liver fat. The effects of anterior pituitary preparations on the ketonuria and liver fat are given in table 1. The results indicate that in general an increased ketonuria is as-

sociated with the presence of excessively large amounts of fat in the liver. This relationship does not appear to be quantitative, and is in agreement with the results obtained by Best and Campbell (9) in fasted rats, who pointed out that wide discrepancies may exist in the magnitude of the ketonuria and the accumulation of liver fat.

Most of the ketogenic activity is associated with the protein fractions (fractions A3 and B3). Heating in an alkaline medium (N/10 NaOH) caused practically complete inactivation (fractions A6 and B4). Heating in an acid medium (N/10 HCl for 30 minutes) resulted in no apparent loss of activity. Thus the ketogenic potency in the protein fractions such as A3 and B3

TABLE 3. THE EFFECT OF PITUITARY PREPARATIONS ON BODY FATTY ACIDS OF MICE (dba)
(Fasted 48 hours)

	Sex	Fatty acids per 100 gm. mouse	Fatty acids per 100 gm. mouse		% fatty acid distribution	
			Liver	Remainder of mouse	Liver	Remainder of mouse
		gm.	gm.	gm.		
Saline: 1.5 cc. in 48 hr.	F	1.15	0.14	1.01	11.9	88.1
Saline: 1.5 cc. in 48 hr.	F	1.07	0.14	0.93	12.7	87.3
Saline: 1.5 cc. in 48 hr.	F	1.03	0.14	0.92	10.5	89.5
A 3: 0.3 gm. in 48 hr.	F	0.87	0.17	0.70	19.6	80.4
A 3: 0.3 gm. in 48 hr.	F	0.98	0.20	0.78	20.5	79.5
A 3: 0.3 gm. in 48 hr.	F	0.88	0.19	0.69	21.6	78.4
622 (Jan. 17): 1.0 gm. in 48 hr.	F	0.79	0.11	0.68	12.6	87.4
622 (Jan. 17): 1.0 gm. in 48 hr.	F	0.81	0.09	0.72	11.0	89.0
622 (Jan. 17): 1.0 gm. in 48 hr.	F	0.82	0.08	0.74	9.7	90.3
622 (Jan. 17): 0.5 gm. in 48 hr. + A 3: 0.15 gm. in 48 hr.	F	0.86	0.09	0.77	9.4	90.6
622 (Jan. 17): 0.5 gm. in 48 hr. + A 3: 0.15 gm. in 48 hr.	F	0.75	0.05	0.70	6.7	93.3
622 (Jan. 17): 0.5 gm. in 48 hr. + A 3: 0.15 gm. in 48 hr.	F	0.71	0.09	0.62	12.7	87.3

compares quite favorably with similar preparations that have not been heated (4).

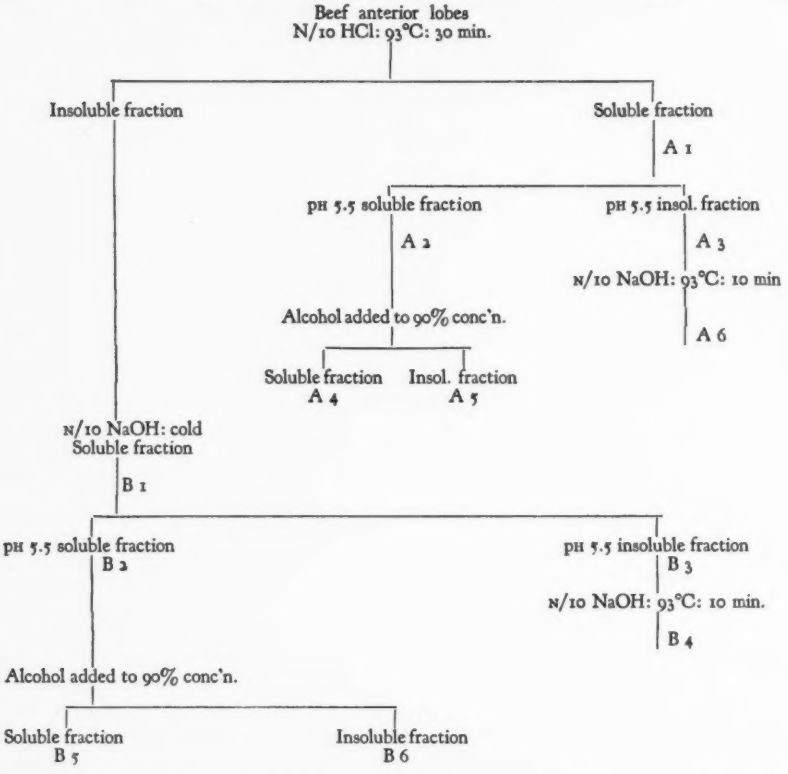
A certain amount of ketogenic activity was found present in the isofiltrates following heating of active protein fractions in an acid medium (fractions A2 and B2). The active substance is precipitated from such isofiltrates by high concentrations of alcohol (fractions A5 and B6). Apparently hot acid extraction of fresh anterior lobe tissue (fraction A1) will not remove the whole of the ketogenic substance. The cold alkaline extract of the residues (fraction B1) still showed a certain amount of activity. A point of interest is that the isofiltrate of the latter fraction (fraction B2) showed an activity comparable to that of the former (fraction A2).

It has been our experience that ketogenic activity is present in any protein fraction irrespective of whether such fractions were obtained in the first instance by acid or alkaline extraction. Similar results were obtained by the use of either an aqueous or alcoholic medium provided the alcohol concentration did not exceed 80%. Heating up to one hour in an acid (N/10

HCl) medium resulted in very little loss of ketogenic activity. Further heating, however, resulted in a gradual loss of potency.

The effect of pituitary preparations on body fatty acids in mice. In tables 2 and 3 results are given of the effect of various pituitary preparations on the total body fatty acids in mice (dba). Results are also given on the distribution of fatty acids between the liver and the other tissues. Preparations A_3

FIG. 1. METHOD OF FRACTIONATION OF ANTERIOR PITUITARY GLAND TISSUE



and B_3 possessed considerable ketogenic potency (table 1). Neither of these preparations when tested in rabbits gave the specific metabolic hormone response, nor did they antagonize the hyperglycemic response to adrenalin. Preparation 622 showed only a slight ketogenic activity. In rabbits, however, 0.5 gm. equivalents of fresh tissue produced in 4 hours following subcutaneous injection a +26.5% rise in oxygen consumption. At the same time an average drop in R.Q. of 0.13 was observed (3 rabbits). No appreciable antagonism to adrenalin hyperglycemia has been observed with this extract. Finally, preparation H_4 showed in rabbits both an antagonism to adrenalin hyperglycemia and an immediate metabolic response as shown in another communication (8). Only a small amount of ketogenic substance was present.

The ketogenic preparations A_3 and B_3 produced in 24-hour fasted mice no appreciable loss of total body fatty acids, but they produced considerable infiltration of fatty acids into the liver (table 2). The latter is in agreement with the results given in table 1 and with those of previous investigators (9, 10, 11). On the other hand, in the 48-hour fasted animals preparation A_3 produced an increase in liver fat, and an appreciable loss of total body fatty acids (table 3). These results are in agreement with those of Best and Campbell (12) who recently reported that 3-day fasted rats injected with their pituitary preparations had 6% less fat than the controls. At the same time they pointed out that owing to the fat concentration in liver and kidneys, the depot fat showed an even more significant fall. It may be pointed out that the preparations used by these authors may well have contained the pituitary factor responsible for an immediate metabolic response.

Preparations 622 and H_4 produced a marked loss of total body fatty acids, associated, in the 24-hour experiment, with a low liver fatty acid content (tables 2, 3). Since the latter extract showed an immediate metabolic stimulating effect and an antagonism to adrenalin hyperglycemia while the former showed only the metabolic effect, we may suppose that the observed decrease in fatty acids content was probably due to the metabolic stimulant.

It may be that primarily pituitary ketogenic extracts produce a mobilization of depot fat. At the same time the increased concentration of fatty acids in the liver may act as a stimulant to remove them either by direct oxidation or some other mode of transformation. On the other hand the low liver fatty acids observed in the 24-hour fasted animals treated with the specific metabolic stimulant may indicate that such preparations stimulate the oxidation of fatty acids. The reduction in liver fat may act secondarily as a stimulant to the mobilization of depot fat. These observations suggested that the administration of ketogenic preparations together with extracts containing the specific metabolic stimulant might produce an enhanced loss of total body fatty acids in fasted mice. This proved to be the case (table 3).

It is not possible at present to decide whether the specific metabolic principle primarily restrains carbohydrate oxidation (13) and thus forces the animal to depend on its fat supplies for energy needs, or whether the decreased carbohydrate oxidation is secondary to an increased fat catabolism (14). Another theoretical possibility which must be considered is gluconeogenesis from fat (15). This is of interest since Soskin et al. (16) have postulated a deficiency of this mechanism in hypophysectomized dogs to explain various experimental findings.

SUMMARY

A study has been made of the ketogenic properties of various anterior pituitary fractions. Points of special interest arising out of these experiments are: association of the ketogenic activity with protein fractions; thermostability of the ketogenic substance over a wide pH range which is confined to the acid side; presence of ketogenic substance in isofiltrates obtained after heating active protein fractions in an acid medium; presence of ketogenic

activity in the precipitate thrown down with alcohol from such isofiltrates.

In 24-hour fasted mice (dba) active ketogenic preparations bring about a fatty infiltration of the liver. Total body fat does not show an appreciable change. In 48-hour fasted animals the high liver fat is associated with a low total body fat. Preparations containing the specific metabolic stimulant produce an appreciable loss of total body fat. This is associated with a low liver fat content in 24-hour fasted animals. Preliminary experiments on the administration of ketogenic preparations together with the specific metabolic stimulant show an enhanced loss of total body fat in mice.

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FURTHER STUDIES ON THE ANTAGONIST TO ADRENALIN HYPERGLYCEMIA IN PITUITARY EXTRACTS

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IN A PREVIOUS communication (1) reference was made to the antagonism shown by certain pituitary preparations to adrenalin hyperglycemia in rabbits. The close association of this with certain other physiological properties such as an immediate metabolic response (2), the glycotrophic (3) and the glycostatic (4) effects, was pointed out. Recently (5) we have shown that the substance responsible for the antagonism to adrenalin hyperglycemia can be differentiated from the others.

The nature of the substance in posterior pituitary extracts which inhibits adrenalin hyperglycemia has been further investigated in rabbits and other animals and the results obtained are herein reported.

MATERIALS AND METHODS

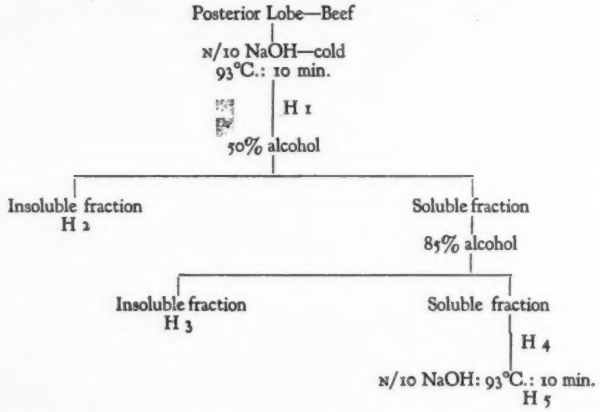
The antagonism to adrenalin hyperglycemia was tested in fed rabbits weighing from 2 to 3 kg. Forty-five minutes after the subcutaneous injection of the extract (15 minutes when extracts were given intravenously) 0.1 mg. adrenalin was injected subcutaneously. Blood sugar determinations, using Somogyi's (6) modification of the Shaffer-Hartman method, were carried out every half hour during a 6-hour period. When the adrenalin was administered intravenously, blood sugar determinations were carried out every 15 minutes during a 3-hour period. Somewhat similar tests were also carried out on other animals (one female dog, 3 female monkeys (*Macaca mulatta*), male hooded rats and male dba mice).

The animals were fasted for the duration of the experiment, and tests carried out at the same hours each test day. All animals were given at least a 7-day rest period between tests. Each animal served as its own control except in the experiments carried out on rats and mice. The rats and mice were disposed of following a single bleeding. Each point on the curve represents the average of 6 determinations. Saline and muscle extracts were used as the control preparations. The O_2 consumption and the respiratory quotients were estimated in rabbits by a modified open Haldane method in a constant temperature room, as previously described by O'Donovan and Collip (2). Most extracts used attained their maximum effect from 2 to 4 hours following the subcutaneous injections. Therefore only the 2 to 4-hour readings are given as the percentage change of O_2 consumption from the

pre-injection level. The antidiuretic potency of extracts was estimated in male hooded rats 8 weeks old, by the method previously described by Burn (7). Oxytocin activity was determined on the guinea pig uterus by the method of Dale and Laidlaw according to Burn (8). All the extracts used, except K1 and K2 were prepared from carefully dissected fresh posterior lobes of beef pituitaries. The methods of preparation are given below.

Extract A was prepared from an absolute alcohol extract of dried glands; 25 gm. dissected lobes were extracted 3 times, 500 cc. alcohol being used for each extraction. Extracts B, C1, D and E were 90, 80, 70 and 60% alcohol extracts of fresh glands respectively—3 successive extractions of 25 gm. fresh tissue with 500 cc. alcohol. Extract C2 was prepared from combined successive extracts (7 in all) with 80% alcohol. Extract F1 was made by extracting fresh tissue with 0.25% hot acetic acid, while extract F2 represents the fraction after heating for 10 minutes in a boiling-water bath in an N/10 NaOH medium. Extract F3 was a cold water extract of 25

METHOD OF FRACTIONATION OF POSTERIOR PITUITARY GLAND TISSUE



gm. fresh glands (3 successive extractions with 500 cc.). In order to decrease the toxicity, preparations A, B, C1, C2, D, E and F3 were kept on a boiling-water bath for 10 minutes in an N/10 NaOH medium prior to use. The method of preparation of extracts H1, H2, H3, H4 and H5 is given in the outline. Extract H6 was the cathode dialysate of preparation H1. Extracts K1 and K2 represent fractions H1 and H4 in the outline respectively, but were prepared from carefully dissected fresh anterior lobes of beef pituitaries. H4-trypsin and H4-pepsin represent fraction H4 treated with trypsin and pepsin (gastric juice from Pavlov pouch dog)—48 hours digestion at 39°C. with trypsin in a pH 8.5 medium and with pepsin in a pH 3 HCl medium. The enzyme activity was checked by the Mett's tube method (9). Extract L was a simple watery suspension of pituitary colloid which had been obtained in the course of dissection of pituitaries of cattle. It was heated on a boiling-

water bath for 10 minutes in an N/10 NaOH medium prior to use. Alcohol present in any of the extracts was removed in vacuo before injection.

RESULTS AND DISCUSSION

A brief summary of the results obtained is given in table 1. The degree of antagonism of adrenalin hyperglycemia is expressed as the percentage response taken at the peak of the blood sugar curve in comparison with the normal control response with adrenalin alone in the same animal. The time of the peak of the blood sugar curve following adrenalin administration may vary in different animals within certain limits (about 30 minutes). The blood sugar curves obtained in two typical experiments are shown in figure 1.

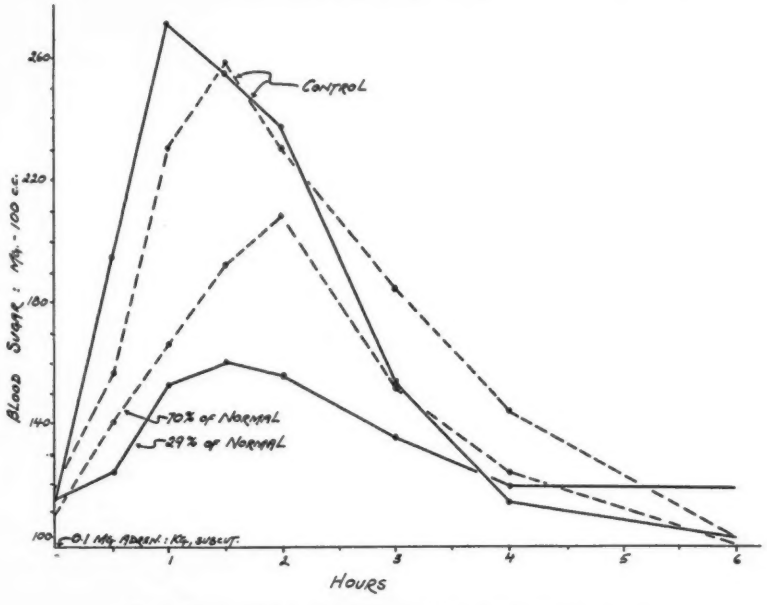


Fig. 1. The antagonism shown by posterior pituitary extracts to adrenalin hyperglycemia in 2 rabbits.

There would appear to be little doubt, from a study of the inhibitory response of the various extracts tested, that the substance causing antagonism of adrenalin hyperglycemia is not of anterior lobe origin. It is of interest that this antagonistic action is not dependent on the mode of administration of either the adrenalin or of the pituitary extract (table 1). From the fractionation studies that have been made it may be concluded that the most complete extraction of the tissue is obtained by the use of large volumes of dilute aqueous alkali. Some activity can be demonstrated in original alcoholic extracts but the residues from such extractions still contain the major part of the active principle. In view of the great toxicity of cold dilute acid

TABLE 1. THE EFFECT OF PITUITARY PREPARATIONS ON ANTAGONISM TO ADRENALIN HYPERGLYCEMIA AND UPON METABOLIC RATE

Preparation	Antagonism to adrenalin hyperglycemia in rabbits		Metabolic response in rabbits 24 hours following injection			Anti-diuretic	Oxytocin
	Dose gm. original tissue subcut.	Increase of blood sugar as % of normal response	Dose gm. original tissue subcut.	% increase in O_2 consumption	Respiratory quotient change	int. units per gm. original tissue	int. units per gm. original tissue
A	1.8	75				abt. 0.05	
	3.2	51					
B	0.7	79				0	
	0.8	79					
C 1	0.7	67				0	0
	0.8	57					
	0.8	76					
C 2	0.7	22				0	6
	0.6	58					
	0.7	40					
D	0.7	116				0	
	0.8	95					
E	0.7	67				0	
	0.8	112					
F 1						264	
F 2	0.7	91				0.9	14
	0.7	76					
	0.8	89					
F 3	0.6	43					
	0.8	69					
H 1	0.6	37	0.5	20.7	0.81–0.74	4	12
	0.6	28	0.5	24.6	0.99–0.82		
	0.7	40					
H 2	0.6	79	0.5	1.0	0.83–0.80	2	<2
	1.4	100					
	1.6	79					
H 3	0.6	108	0.4	21.0	0.93–0.83	1	
	1.2	37					
	1.4	48					
H 4	0.4	104	0.6	25.0	0.97–0.78	1.2	10
	0.8	29	0.6	26.5	0.95–0.83		
	1.6	24					
	1.6	29					
H 5	0.6	97				0	
	1.6	98					
H 6	0.6	29	0.25	9.1	0.89–0.81	1.9	
	0.5	9					
	0.4	39					
K 1	0.7	92	0.5	9.7	0.91–0.82	0	
	0.8	108					
K 2	0.7	82	0.5	11.1	0.90–0.82	0	
	0.8	98					
	1.2	99					

TABLE 1.—*Continued*

Preparation	Antagonism to adrenalin hyperglycemia in rabbits		Metabolic response in rabbits 24 hours following injection			Anti-diuretic	Oxytocin
	Dose gm. original tissue subcut.	Increase of blood sugar as % of normal response	Dose gm. original tissue subcut.	% increase in O_2 consumption	Respiratory quotient change	int. units per gm. original tissue	int. units per gm. original tissue
H 4 trypsin	1.0	79	1.6	6.7	0.85–0.85		
	1.0	81					
H 4 pepsin	1.0	54	1.6	17.6	0.87–0.83		
	1.0	50					
L	0.18	74					
	0.22	69					
	0.26	53					
	0.05 mg. adrenalin kg.: intraven.						
H 1	0.8	29					
H 3	1.4	52					
	1.2[1]	45					
H 4	1.0	42					
	1.0	47					
	1.2	45					
	1.4	45					
	1.0[1]	47					
	1.0[1]	21					
	1.0[1]	45					
	1.4[1]	17					

[1] Intravenous injection.

extracts of posterior lobes, it is impossible to tell just how efficient acid reagents may be for extraction.

The close association of the metabolic stimulant with the antagonist to adrenalin hyperglycemia is well illustrated in fractions H1, H2, H3 and H4. Both factors are dialyzable on electrodialysis and pass to the cathode cell (fraction H6), in agreement with our previous findings (1, 5). On the other hand, fractions K1 and K2, prepared from carefully dissected anterior lobes, give only the specific metabolic response. This, together with the instability of the antagonist to prolonged heat treatment in an acid medium (N/10 HCl) as compared to the stability of the metabolic stimulant to such treatment (5), establishes the separate identities of the substances responsible for these two effects. The resistance to pepsin and the evidence of destruction by trypsin shown by the antagonist to adrenalin hyperglycemia is similar to the behavior of the specific metabolic principle to these two proteolytic enzymes. The assays of different preparations of the adrenalin hyperglycemia antagonist for oxytocin and antidiuretic properties fail to show any parallelism between the former and either of the two latter effects. These results suggest that the substance responsible for antagonism of adrenalin hyperglycemia is separate and distinct from oxytocin and vaso-

pressin. The instability of the antidiuretic substance to heat in an alkaline medium is well illustrated with fractions F1, F2, H4 and H5. It is of special interest that the simple extract of pituitary colloid (extract L) proved to be highly potent in the relation to antagonism of adrenalin hyperglycemia.

TABLE 2. THE EFFECT OF PITUITARY PREPARATIONS ON ANTAGONISM TO ADRENAL HYPERGLYCEMIA

Animal	Sex and weight	Extract	Dose gm. equivalent fresh tissue subcutaneous	Antagonism to adrenalin hyperglycemia	
				Adrenalin mg per kg. subcutaneous	Increase of blood sugar as % of normal response
	kg.				
Dog	♀ 6.70	H 4	2.0	0.1[1]	28
		H 4	2.0[1]	0.1[1]	25
Monkey	♀ 4.20	H 4	2.0	0.1	40
	♀ 3.51	H 4	2.0	0.1	31
	♀ 3.64	H 4	2.0	0.1	54
Rats	♂	H 4	0.8	0.2	48
Mice	♂	H 4	0.06	0.2	53

[1] Intravenous injection.

The results shown in table 2 show that the preparation H4 antagonizes the hyperglycemic effect of adrenalin in dogs, monkeys, rats and mice as well as in rabbits.

Stenström (10) and others (11, 12, 13) were the first to show that adrenalin hyperglycemia may be reduced considerably by the injection of posterior pituitary extracts. Blotner and Fitz (14) first suggested that this perhaps is due to insulin liberation. This is of particular interest since LaBarre (15) showed from cross-circulation experiments in dogs that posterior pituitary extracts cause a liberation of insulin by stimulating the pancreas directly. Under such conditions one would expect that preparations showing antagonism to adrenalin hyperglycemia should by themselves produce a lowering of blood sugar. It has been our experience that the subcutaneous injections of fractions H1 and H4 into either fed or fasted rabbits show no well defined effects on blood sugar. Intravenous injections produced a slight rise of blood sugar (10 to 15 mg.) during the first 15 minutes. A hypoglycemic effect, however, has never been observed.

SUMMARY

Evidence has been presented to show that certain posterior pituitary preparations produce on administration to mice, rats, rabbits, monkeys and a dog, an antagonism to adrenalin hyperglycemia. A number of the properties of the substance responsible for the antagonism are given. This substance can be differentiated from the factor responsible for the immediate metabolic response. The evidence available does not indicate a relationship of this substance to other well-known pituitary principles.

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THE EFFECT OF ANDROGENS ON THE SURVIVAL OF ADRENALECTOMIZED RATS[1,2]

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THERE IS considerable evidence supporting an adreno-gonadal relationship (1). The similarity in embryological development and the histological changes produced in the adrenal following gonadectomy are well known. Many workers (1) report that the survival of adrenalectomized female animals is longer when the operation is performed during estrus or pregnancy. Furthermore, the gonadal hormones and the adrenal cortical hormones are all steroids (2). It is naturally suggested, therefore, that sex hormones might be used by the body to fulfill the vital function of the adrenal cortex.

Since little else is known of the function of corticosterone than that it suffices to keep adrenalectomized animals alive, the only crucial test of the substitutional properties of the sex hormones available at present is their effect upon survival time of such animals. The month-old adrenalectomized rat has served as subject of many analogous experiments (3–6).

METHODS

An attempt was made throughout this work to control all factors known to produce variations in the survival. The rat room was thermostatically controlled so that the temperature could not fall below 76° C. and never went above 80° during the course of the experiments.

Young male rats of our colony of the Wistar strain weighing 40 to 60 gm. were used. Purina dog chow and water, both ad libitum, were the only food furnished from weaning till death.

Grollman and many others (1) have reported that rats survive adrenalectomy longer if barbital anesthesia is used in preference to ether. For this reason all rats were anesthetized by intraperitoneal injections of sodium pentobarbital (nembutal) 4 mg./100 gm. body weight, from a solution containing 6 mg./cc. Occasionally it was necessary to supplement this anesthesia with a little ether. We found no evidence that the supplementary anesthesia used in these cases altered the survival time.

The technic followed in extirpating the adrenals was identical to that of Schultzer (5). Since the rat is highly resistant to infection, alcohol asepsis was sufficient to give clean healing of the incisions. Especial care was taken to remove the surrounding perirenal fat and never to touch the gland.

[1] This investigation was supported by a grant from the International Cancer Research Foundation administered by Prof. John R. Murlin.

[2] The data in this paper were presented on April 2, 1938 before the American Physiological Society at the Federation of American Societies for Experimental Biology meeting at Baltimore, Md.

After the operation the animals were placed in wood shavings and allowed a day to recover. Any animal not showing complete recovery was discarded. Body weights were recorded each morning at the same hour and the survival noted to the closest half day.

The androgens were injected subcutaneously in sesame oil solution twice daily with an interval of approximately 10 hours. The control groups were treated in an identical manner with sesame oil. The volume of solution was with a few exceptions 0.1 cc. Two control groups received no injections.

Materials.[3] An androgenic extract of male urine was prepared by the method of Kochakian and Murlin (7). The neutral lipoid fraction has been shown to contain androsterone (8), dehydroandrosterone (9), possibly isoandrosterone (10) and the inactive steroid epietiocholandiol (11). Assay of this extract by the capon method of Gallagher and Koch (12) gave 125 international units (I.U.) per liter of original urine or 625 I.U. per cc. of oil solution. The urine extracts also are highly potent in the castrate rat test (13).

Androstenedione, a synthetic androgen, was selected because it differs only by one less oxygen atom from Reichstein's adrenosterone (14), an androgenic substance extracted from the adrenal cortex. The androgenic potency of androstenedione in the capon test is the same as that of androsterone (15).

Since testosterone is thought to be the product of the interstitial cells of the testis which are so closely related histologically to the adrenal cortex, it is pertinent to consider its replacement value in adrenal cortical deficiency. This compound is five[4] to seven times more active in the capon test than the most active urinary component, androsterone (15). The activity of testosterone in the rat test is enhanced markedly when it is given in the form of an acetate or propionate ester (13, 16).

RESULTS

The experiments and the nature of their treatment are presented in concise form in the table-graph, figure 1. The average survival of the 2 control groups is 8.7 days which compares favorably with other recent studies (1). Handling and trauma are deleterious factors known to shorten the survival of adrenalectomized animals. Since the rats receiving injections twice daily would be handled approximately two times more than the untreated, and because it might be said that the subcutaneous injections would introduce a traumatic factor, 2 groups of control animals were injected with sesame oil. The average survivals were 7.8 and 6.7 days, or an average of 7.1 days for the 2 groups. This decrease of 1.6 days must be attributed to the handling and treatment.

In spite of the fact that androstenedione was given in doses of 0.5, 1.0 and 3.0 mg., there is no indication of a beneficial effect. On the other hand, the rats treated with urine extract show peculiarly significant results. A group receiving the equivalent of 0.4 liter urine had a survival of 6.2 days which is quite comparable to the controls. When the dose was increased to

[3] The crystalline androgens used in this study were supplied through the courtesy of Dr. E. Schwenk of the Schering Corporation and Dr. E. Oppenheimer of Ciba Pharmaceutical Products.

[4] In our laboratory we find this compound to be 5 times that of androsterone.

1.2 liters per day the survival decreased to 4 days. On further increasing the dose the survival time decreased to 2.7 days. Thus, there is a definite lowering of the survival time as the dosage is increased.

When testosterone esters are given survival time shows a slight decrease which, however, is apparently not proportional to the dose. The average survival is 6.1 days for a 0.28 mg. per day dose, 5.9 days with a daily dose of 1 mg. and 3.6 when the dose is 2.5 mg. Treatment of a group of female rats with 5 mg. per day of testosterone propionate resulted in a survival time of 3.8 days as compared to 8.7 days for a similar group of controls, a decidedly toxic effect.

FIG. 1

EFFECT OF ANDROGENS ON SURVIVAL TIME OF ADRENALECTOMIZED RATS

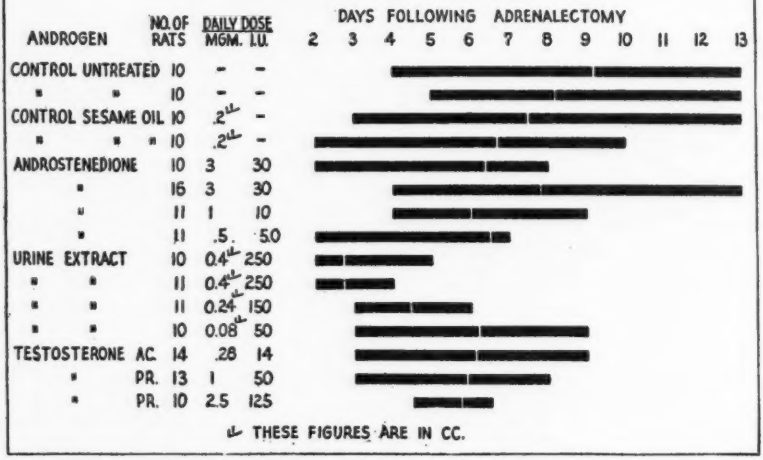

ANDROGEN
NO. OF RATS
DAILY DOSE MGM. I.U.
DAYS FOLLOWING ADRENALECTOMY
2 3 4 5 6 7 8 9 10 11 12 13
CONTROL UNTREATED 10 - -
" " 10 - -
CONTROL SESAME OIL 10 .2cc -
" " " 10 .2cc -
ANDROSTENEDIONE 10 3 30
" 16 3 30
" 11 1 10
" 11 .5 5.0
URINE EXTRACT 10 0.4cc 250
" " 11 0.4cc 250
" " 11 0.24cc 150
" " 10 0.08cc 50
TESTOSTERONE AC. 14 .28 14
" PR. 13 1 50
" PR. 10 2.5 125
cc THESE FIGURES ARE IN CC.

Figure 2 illustrates the relationship of toxicity to activity of the androgens in the capon test. Although the number of points are few, there is nevertheless an apparent direct relationship.

DISCUSSION

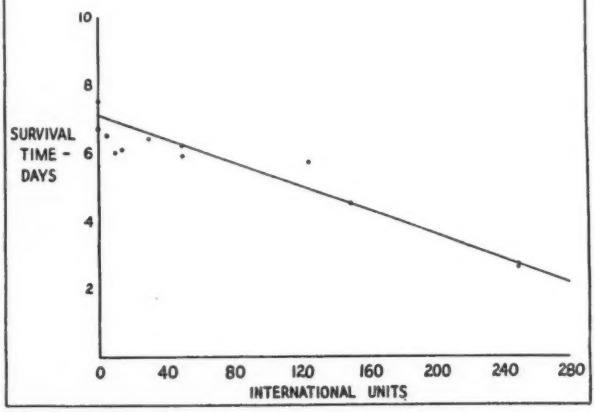
It is quite clear from the results of this study that androgens cannot be substituted for the life-maintaining principles of the adrenal cortex. On the contrary these substances are toxic to the adrenal cortical deficient animal.

Loeb (17), Harrop (18) and their respective coworkers have presented the view that the principal function of the adrenal cortex is the regulation of sodium and water content of the blood through its influence on the renal excretion of these substances. Thorn and Harrop (19) found that several sex hormones including testosterone propionate were able to cause retention

of sodium in normal dogs. Since androgens in our experiments not only failed to maintain life but decreased the survival time of adrenalectomized rats, we are led to wonder if the specific property of sodium retention is the vital function of the adrenal cortex. The recent report (20) of the presence of a sodium-retaining factor in the adrenal cortex independent of corticosterone presents more direct evidence on this subject. The results of Jiminez-Diaz' work (21) are also a challenge to the sodium maintaining theory for the adrenal cortex. This worker finds that cortical deficiency renders the kidney asthenic and incapable of producing ammonia. This leads to an increased sodium excretion in an attempt to maintain acid-base balance.

FIG. 2

THE RELATIONSHIP OF SEX ACTIVITY TO TOXICITY



There is as yet no definite explanation of the toxic properties of androgens in the adrenalectomized rat. It seems logical, however, to tie it in some manner to the adrenal deficiency. It is known (1, 22) that the liver as well as the kidney of an adrenalectomized animal undergoes profound changes with respect to its normal physiological functions. Furthermore, it is becoming apparent (23–29) that injected androgens are not excreted as such but at least in part in other form(s) and in a conjugated state. The malfunction of the liver, therefore, would seriously impede the proper elimination of these substances by the body. Another view is presented by the findings of Schacher et al. (30). These workers found that while adrenalectomy produced all the other symptoms of the 'alarm reaction,' it prevented thymus involution. This, however, could be brought about when steroid sex hormones but not other steroids were administered. The effect, furthermore, was related to the physiological dose. In our results, too, the toxicity more nearly parallels the sex activity of the material injected than the quantity or the chemical nature of the substances.

SUMMARY

The month-old male adrenalectomized rat has an average survival time of 8.7 days which is decreased to 7.1 days by injecting twice daily with 0.1 cc. of sesame oil. The administration of androgens—urinary androgenic extract, 50, 150, 250 and 250 I.U.; androstenedione 5, 10, 30 and 30 I.U.; testosterone acetate 14 I.U. and testosterone propionate 50 and 125 I.U. per day—resulted in a marked decrease in survival time which was roughly proportional to their physiological activity. At 250 I.U. (urinary extract) the survival time was decreased to 2.7 days.

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THE RELATION OF THE ADRENALS TO THE RETARDED HAIR GROWTH IN UNDERFED ALBINO RATS

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A PREVIOUS PAPER (1) contained statistics showing to what extent hair growth in rats was affected by underfeeding. When the quantity of feed was reduced so that little body weight was gained the body increased in length, but hair growth was delayed. Since some skeletal growth occurred in these underfed animals, building factors were present in the body. It would, therefore, seem that in all probability the retarded hair growth was due to the deviation of factors from the hair follicles.

Since a precocious hair growth occurred in well fed adrenalectomized rats (2), and adrenals hypertrophied in underfed animals (3, 4), it occurred to the authors that the adrenal might be responsible for this retardation of hair growth upon underfeeding. Investigations were accordingly undertaken in underfed animals to see if adrenalectomy would induce hair growth and if there were any indications that the adrenals changed and caused the retarded effect.

The present investigations show that the hair buds begin proliferating cells for new hairs in underfed animals often within 40 hours after adrenalectomy, despite the fact that the rats are in a very poor physical condition. A study of the adrenals of undernourished rats shows that the glands hypertrophy and that there are histological changes indicative of great secretory activity in the cortex. About the time that the hair finally grows in the underfed rats, the adrenal appears to retain its secretion and many cells are restored to the cortex from the capsule. It seems quite probable that these changes in the adrenal during inanition have something to do with the retarded hair growth.

The Effect of Adrenalectomy on Underfed Rats

Rats were isolated when 22 days old and were thereafter fed Purina Dog Chow which was finely ground to minimize the loss from the feeding cups. Each animal received daily 2 gm. of this feed in the morning and 2 gm. in the evening. Rats gained very little weight on this quantity of food.

Usually at the age of 23 days the hair was removed from the backs of the rats with sodium sulphide. Several individuals of the litter were then adrenalectomized via the dorsal approach while other litter mates which were to serve as controls were subjected to a mock adrenalectomy. Three per cent

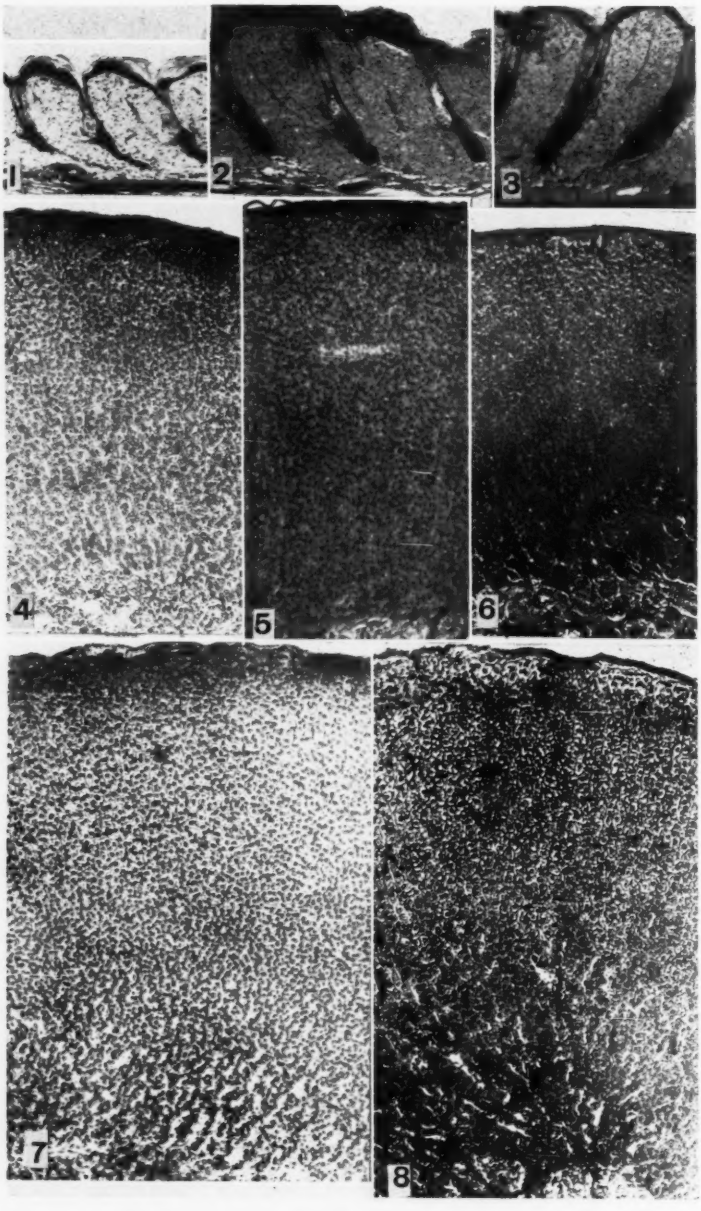

Fig. 1. Skin from 48-day-old rat, underfed since weaning time (22 days). ×60. Fig. 2. Skin from 48-day-old rat, underfed since weaning time and adrenalectomized at the age of 46 days. ×60. Fig. 3. Skin from 49-day-old rat, underfed since weaning time and adrenalectomized at the age of 46 days. ×60. Fig. 4. Section of adrenal of 22-day-old rat. The z. glomerulosa is sharply demarcated from the z. fasciculata. Little vacuolization is noted in the cells of the cortex

NaCl solution was kept at the disposal of all adrenalectomized animals. Most adrenalectomized animals failed to eat their daily allowance of 4 gm. of food, while a similar quantity in the cage of a control animal was quickly consumed.

The second growth of hair appeared on the backs of the underfed adrenalectomized rats when they were 32 days old, which is about the same time as when the rats are adrenalectomized on the 22nd day and have quantities of food accessible. This similarity in the time of growth is to be expected, however, since less food is necessary to satisfy the adrenalectomized animals.

When underfed rats were adrenalectomized at the age of 35 days, the hair buds began activity in some instances as soon as 40 hours (fig. 2) after the operation and by 72 hours (fig. 3) growth of the hair bud had usually occurred. About 8 days after adrenalectomy the new growth of hair appeared very evenly over the dorsum externally despite the very poor physical condition of the rat. Underfed litter mates failed to produce hair for several weeks (average age of 63 days in these experiments) and then it usually grew slowly and in patches.

In well-fed animals hair growth occurs as a wave. It begins on the venter, then passes dorsally, then anteriorly and posteriorly over the back, the growth occurring last between the ears and at the base of the tail. In adrenalectomized animals, however, growth occurs about the same time on both venter and dorsum.

It would, therefore, seem that the adrenal is at least partly responsible for the retardation of hair growth in these underfed animals. Since hair growth starts so soon after adrenalectomy, it scarcely seems possible that other endocrine glands could be influenced so suddenly by adrenalectomy that they could cause this sudden growth. There is evidence that the permeability of capillaries increases (5) after adrenalectomy and it would seem quite plausible to think that this sudden growth of hair after adrenalectomy is due to the rapid passage of materials through the capillary wall to the hair buds. Experiments are being conducted to determine if this is true.

Changes in the Adrenals of Underfed Animals

Animals used for studying the effects of underfeeding on the size and histological changes in the adrenal were litter mates and usually of the same sex. Two individuals of the litter were killed at the time of weaning or at the age of 22 days and the other rats of the litter, usually 4 or 5 in number, were isolated and the hair was removed from their backs with sodium sulphide. Usually 2 of these 4 rats served as controls and were supplied with an excess of finely ground Purina Dog Chow while the other two

×110. Fig. 5. Adrenal of normal 35-day-old rat. Note that glomerulosa and fasciculata are indistinctly separated, and that the cells of the cortex contain more vacuoles than the younger rat. ×110. Fig. 6. Adrenal of an underfed 35-day-old rat. The extent of z. glomerulosa cannot be determined and the cells of the z. glomerulosa and outer fasciculata are greatly vacuolated. ×110. Fig. 7. Adrenal of underfed rat (63 days old) which is producing hair. Note that the extent of the glomerulosa is distinct and its cells are less vacuolated. ×110. Fig. 8. Adrenal of normal 65-day-old rat. Note the vacuolization of the cells of the z. glomerulosa and z. fasciculata. ×110.

litter mates were kept from gaining much weight by feeding them 2 gm. of the chow in the morning and 2 gm. in the evening. One control and one underfed animal were killed when 35 days old. Another underfed individual and its well-fed litter mate were usually killed when the hair appeared on the back of the underfed animal.

After killing the animals the adrenals were carefully removed, weighed and preserved in various fixers. The glands were then imbedded after the usual dehydration and infiltrating procedure, sectioned and stained in hematoxylin and eosin.

The adrenals of animals at weaning time as shown in table 1 make up .019% of the body weight in males and .023% in females. In normal animals, 35 days old, the adrenals have increased in absolute weight 60% in the males and 73% in females. They, however, now constitute only .018% of the total body in males and .020% in females since the body has made a slightly greater gain proportionately than the adrenals. Despite the fact that body weight is held nearly constant the adrenals of underfed animals, 35 days old, also continue to increase in absolute weight (41% in males,

TABLE 1. BODY WEIGHT AND ADRENAL WEIGHT OF UNDERFED AND NORMAL RATS

No. of rats	Body wt. on 22nd day Mean and S.D.	Treatment	Age when killed	Body wt. when killed Mean and S.D.	Adrenals wt. when killed Mean and S.D.	Increase of adrenals in abs. wt. over 22nd day	Adrenals per cent of body weight
			Females				
	gm.		days	gm.	mg.		
6	40.1±2.7		22	40.1±2.7	9.3±1.9		.023%
6	40.0±3.5	well-fed	35	81.7±5.0	16.1±2.8	73.0%	.020%
7	44.5±3.2	underfed	35	49.2±3.0	13.9±2.4	49.5%	.028%
1	41.9	underfed	45	44.6	15.6	67.8%	.035%
6	40.9±3.3	underfed	when hair grew Av. 61	56.1±5.7	16.0±2.0	72.1%	.029%
2	32	well-fed	Av. 58	124	36.1	288%	.029%
			Males				
6	39.6±2.2		22	39.6±2.2	9.3±1.5		.019%
6	41.0±4.1	well-fed	35	81.7±11.6	14.9±1.9	60.3%	.018%
9	41.0±2.6	underfed	35	45.1±2.2	13.1±1.8	40.9%	.029%
1	41.0	well-fed	45	99.8	18	93.6%	.018%
4	39.9±1.3	underfed	45	48.8±4.4	14.7±2.5	58.0%	.030%
6	41.2±2.1	underfed	when hair grew Av. 65	61.1±6.6	16.9±1.3	81.7%	.028%
2	34.5	well-fed	Av. 62	180	26.5	185%	.015%

50% in females), and they make up .029% of the body in males, and .028% in the females. Jackson (3), likewise observed such relations between the adrenals and body weight, particularly in underfed females. When the hair finally grows on an underfed animal the adrenal constitutes the same percentage of the body as it does in younger underfed rats. As pointed out by Donaldson (6) normal females have heavier suprarenals than normal male rats.

A histological examination of the adrenal of the normal 22-day-old rat (fig. 4) shows that the rather definitely demarcated zona glomerulosa is about 10 cells deep. The cells of the glomerulosa, containing round or oval nuclei, are arranged in irregular masses which are separated by blood capillaries. Cell boundaries are poorly defined and the cytoplasm, which is scanty, contains fine eosinophilic granules. The zona fasciculata or middle zone consists of cells arranged in columns, usually a single cell in width. The cytoplasm of the cells which have spherical nuclei, is very abundant and is filled with eosinophilic granules and a few vacuoles. The zona fasciculata merges with the zona reticularis, the rather narrow inner zone. The cells of the inner zone are similar to those of the adjacent middle zone, and likewise contain many eosinophilic granules and an occasional vacuole. Irregular cell masses enclosing sinusoidal blood vessels constitute the medulla. The cells are usually not vacuolated and are filled with pale violet granules.

The cells of the adrenals of normal animals, 35 days old (fig. 5), contain more vacuoles than do the cortical cells of 22-day-old rats. An area of the outer zone or glomerulosa may be greatly vacuolated while the cells of another area may be entirely filled with eosinophilic granules. The cells in the outer half of the fasciculata are more vacuolated than the cells of the inner half. Vacuoles appear throughout the cytoplasm of the cells in the outer fasciculata and increase in size with the disappearance of the granular inclusions. The inner zone or reticularis is wider than the similar zone in the 22-day-old rat, and contains an occasional pigment cell. Few changes are observed in the medulla.

Occasionally the differences between the adrenal of an underfed animal, 35 days old, and that of a normal litter mate of the same age are slight. Usually, however, the glomerulosa is not so distinctly demarcated from the fasciculata in the underfed animal (fig. 6). Vacuoles are more numerous in the cells of the glomerulosa and particularly in the cells of the outer part of the fasciculata. The nuclei of the cells in the glomerulosa are usually pycnotic. Eosinophilic granules are less distinct in the cells of the glomerulosa and only an occasional cell in the outer half of the fasciculata contains any granules. The cells in the inner part of the fasciculata are granular and merge with the cells of the reticularis. The zona reticularis of the adrenal in our underfed rats does not show the marked atrophy and hyperemia described by Jackson (7). Pigment masses are seen but even these are not abundant. It seems to us that there is less change in the gland of the underfed animal as the gland is penetrated from the outside. There is, however, some vacuolization and the disappearance of cell inclusions in the medulla.

When the hair starts growing externally in the underfed animals the structure of the cortex (fig. 7) of the adrenal resembles somewhat the conditions found in the 22-day-old normal animal. The glomerulosa has definite limits and its cells contain granules and only a few vacuoles. New cells are added to the glomerulosa from the capsule as described by Zwemer (8). The cells of the fasciculata are smaller, less vacuolated and contain more granular inclusions than the cells of the 35-day underfed rat. The reticularis

is narrow and both it and the medulla closely resemble structurally the same regions of the 22-day-old rat.

The adrenals of underfed animals whose hair is just beginning to grow vary greatly from the glands of control litter mates. The adrenals of the control animals (fig. 8) more closely resemble the adrenals of the normal 35-day-old rat. In the control animals the cytoplasm of the cells of the glomerulosa is scanty, somewhat granular and the cells contain even more vacuoles. The number of vacuoles is also increased in the middle zone.

Osmic acid preparations of the adrenals of underfed animals show that there is a tendency for a more uniform distribution of fat throughout the cortex than there is in the normal animal. In general the lipoidal content of the cortex decreases in the underfed animal.

SUMMARY

Hair buds in underfed rats began active proliferation and the formation of new hair soon after adrenalectomy. Underfeeding induced enlargement of the adrenals and the glomerular zone became wider and indefinite. Secretory granules apparently were released and the cells became smaller. Likewise, the fascicular zone became more extensive and its cells apparently released their inclusions. The reticular zone and medulla were less affected by underfeeding. These conditions resulting from underfeeding seem quite comparable to the changes described by Zwemer (8) when the demand on the adrenal was mild. Observations indicate, therefore, that the gland in our underfed animals hypertrophied and released secretions. It is interpreted that these secretions acted in some way to retard hair growth.

When the hair finally grew in the underfed animal, new cells were added to the glomerulosa from the capsule, the glomerulosa became more definitely demarcated and the granular inclusions of the cortex greatly increased. It would seem, therefore, that a condition was finally reached in the animal when there was no longer the necessity for the adrenal to retard the hair growth.

Since hair growth is induced so quickly after adrenalectomy and the adrenal undergoes hypertrophy and histological changes in the underfed animal, it is believed that the adrenal in some way is partly responsible for the retarded hair growth in inanition.

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PROPERTIES OF OVARIAN EXTRACTS[1]

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FOR A LONG TIME it has been recognized that the ovary is made up of several distinct parts. The corpora lutea, the maturing graafian follicles, the interstitial tissue, and the primary, or immature follicles constitute 4 parts. Investigations have been made to prepare hormones from 3 of these parts. Progesterone has been obtained from the corpora lutea of the ovary (1–4). Extracts from the corpora lutea have also yielded a substance with estrogenic powers (6, 7, 8) which is thought to be estrone (2). Also, relaxin, a substance with specific action on pelvic ligaments, has been reported (1).

Extracts of the portion of the ovary remaining after removal of the corpora lutea and the follicular fluid are frequently spoken of as extracts of the residual ovary. A number of investigators (9, 10) have made such extracts and studied their estrogenic content. For the author (11, 12) the estrogenic content of the residual ovary with and without the corpora lutea has been nearly constant. The residual ovary referred to in this paper is the ovary from which the follicular fluid alone has been removed. The separation of the interstitial tissue from the immature follicles would be practically impossible for the mature ovary contains some fifteen thousand follicles. Finally, although some investigators believe that the interstitial cells are secretory (13, 14, 15), there are some who contend that, in contrast to the interstitial cells of the testes, these cells have no secretory function (16).

Many investigations have been made upon the estrogenic hormone from the follicular fluid (10, 17, 18). In 1935, Doisy and coworkers (19) isolated a minute amount of an active crystalline substance as the m-brom-benzoyl derivative which had from 4 to 8 times the estrogenic activity of estrone. This compound had the chemical and biological properties of reduced estrone. This is the α-estradiol as prepared by Schwenk and Hildebrandt (20) who obtained this compound by reduction of the ketone group of estrone. Girard, Sandulesco and Fridenson (21) also obtained estradiol from the reduction of estrone by metallic sodium and amyl alcohol. Other reduction methods for converting estrone into estradiol have been devised. The preparation of this crystalline estrogenic compound from the ovary has led Doisy to think that estradiol is the principal estrogenic compound of the ovary.

Dorfman, Gallagher and Koch (22) have suggested that the uterine response to the estrogenic compounds affords a method of comparison of the respective activities of such compounds. They used the ratio of the weight of the uterus in milligrams to the body weight less the gut in grams. They have shown the uterine responses to estrone and estriol as well as other substances from the testes. Marlow (23) published such a comparison between estrone and estradiol. This paper, in agreement with the statements of Doisy and others, shows that estradiol produces a much more marked

[1] Contribution No. 232, Department of Chemistry.

hypertrophy of the uterus than estrone. In the present paper is presented a comparison of the residual ovarian extracts and follicular fluid extracts as compared to estrone and estradiol in their effect upon the uterus of immature albino rats. Further properties that may help in identification of the estrogenic activity of these extracts are presented.

Preparation of the Extracts

The residual ovarian extracts were prepared as reported by Groetsema and Marlow (11) and by Marlow and Koch (24). The essential details of the preparation of extracts from the follicular fluid are as follows. The ovaries were obtained from a packing house in a frozen condition. (Reports from the packing company say that the ovaries were frozen solid in 30 minutes after extirpation.) While still frozen, the mature graafian follicles were dissected and the ice (frozen follicular fluid) was placed directly into 95% alcohol. The follicles were then washed out with alcohol to remove all the fluid possible. The follicular fluid-alcohol mixture was then boiled and the precipitated proteins removed by filtration. From this point on to the purified extract the procedure is the same as described for the alcoholic extract in the 2 papers referred to above (11, 24).

Assay of Extracts

The assay for all estrogenic compounds mentioned in this paper was essentially that of D'Amour and Gustavson (9). Briefly the procedure was as follows. Mature, castrated female albino rats were used. They were given a single subcutaneous injection of the extract in olive oil. Vaginal smears were taken at 42, 48 and 54-hour intervals. The absence of all non-nucleated epithelial cells were required for a positive smear. The estrone required 0.84γ and the estradiol required 0.154γ per R.U. (Dr. Gallagher in Dr. Koch's Laboratory of the University of Chicago kindly furnished alcoholic solutions of these 2 substances prepared from the crystalline compounds.)

Response to Residual Ovarian Extracts

Below are summarized the results of the injection of residual ovarian extracts. The dosage in R.U. following the assay suggested by D'Amour and Gustavson (9), the number of animals involved under each dosage, minimum and maximum values, the uterine response as suggested by Dorfman and others (22), and the percentage exhibiting vaginal introitus are shown in table 1.

TABLE 1. RESIDUAL OVARIAN EXTRACTS

R.U.	No. of Animals	Minimum	Maximum	Ratio	Vaginal Introitus %
0.05	7	.40	.54	0.50	0
0.10	7	.63	.84	0.76	0
0.15	4	.83	1.04	0.91	10
0.30	4	1.23	1.44	1.39	100
0.40	4	1.51	1.74	1.65	100
0.50	7	1.81	1.95	1.88	100
0.60	9	1.83	2.12	1.95	100

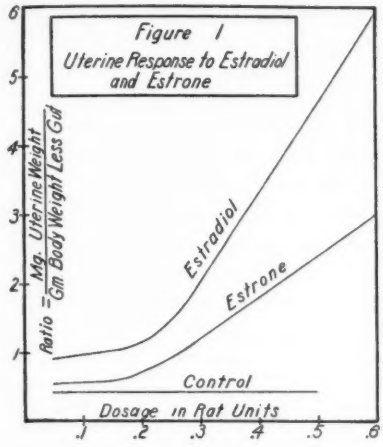
Figure 1
Uterine Response to Estradiol and Estrone
Ratio = Mg. Uterine Weight / Gm. Body Weight Less Gut
Estradiol
Estrone
Control
Dosage in Rat Units
1
2
3
4
5
6
.1
.2
.3
.4
.5
.6

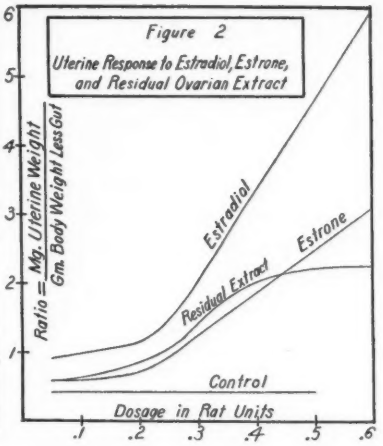
Figure 2
Uterine Response to Estradiol, Estrone, and Residual Ovarian Extract
Ratio = Mg. Uterine Weight / Gm. Body Weight Less Gut
Estradiol
Residual Extract
Estrone
Control
Dosage in Rat Units
1
2
3
4
5
6
.1
.2
.3
.4
.5
.6

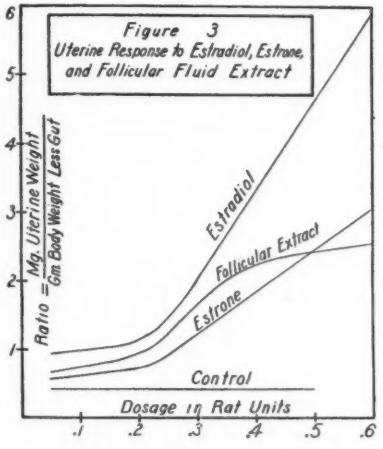
Figure 3
Uterine Response to Estradiol, Estrone, and Follicular Fluid Extract
Ratio = Mg. Uterine Weight / Gm. Body Weight Less Gut
Estradiol
Follicular Extract
Estrone
Control
Dosage in Rat Units
1
2
3
4
5
6
.1
.2
.3
.4
.5
.6

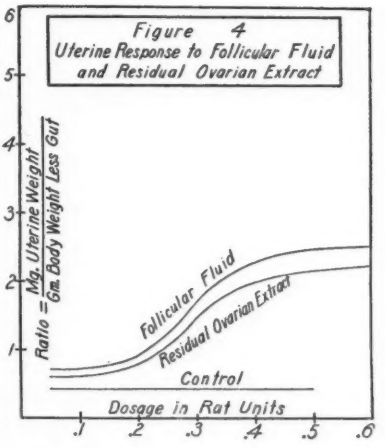
Figure 4
Uterine Response to Follicular Fluid and Residual Ovarian Extract
Ratio = Mg. Uterine Weight / Gm. Body Weight Less Gut
Follicular Fluid
Residual Ovarian Extract
Control
Dosage in Rat Units
1
2
3
4
5
6
.1
.2
.3
.4
.5
.6

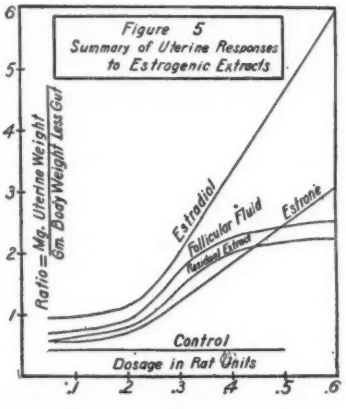
Figure 5
Summary of Uterine Responses to Estrogenic Extracts
Ratio = Mg. Uterine Weight / Gm. Body Weight Less Gut
Estradiol
Follicular Fluid
Residual Extract
Estrone
Control
Dosage in Rat Units
1
2
3
4
5
6
.1
.2
.3
.4
.5
.6

In the graph, figure 1, is shown the dosage in R.U. as abscissa and the ratio of uterine weight to body weight as the ordinate. The curves of estrone, and estradiol were reproduced from a paper by the author (23). It might be observed here that the paper by Dorfman, Gallagher and Koch (22) shows that estriol gave a maximum response at 0.6 R.U. of a ratio of less than one. It is to be observed from figure 2 that the residual ovarian extract follows between the curve of estrone and that of estradiol until 0.4 R.U. has been reached. It then begins to break and at 0.6 R.U. is decidedly below that of estrone. This portion of the curve has been reinvestigated and the results are reproducible.

In table 2 is shown the effects of injection of the extract of follicular fluid.

TABLE 2. FOLLICULAR FLUID EXTRACTS

R.U.	No. of Animals	Minimum	Maximum	Ratio	Vaginal Introitus %
0.1	5	.63	1.06	0.81	20
0.3	6	1.48	1.60	1.51	100
0.4	5	1.94	2.03	2.00	100
0.5	9	2.22	2.60	2.40	100
0.6	11	2.50	2.70	2.58	100

The comparison of this extract with estrone and estradiol using litter-mate controls is plotted in figure 3. It can be observed that the follicular fluid extract gives a curve similar to that of ovarian extracts in that it follows between the curves of estrone and estradiol until it reaches 0.4 R.U. where it breaks sharply to show less response at 0.6 R.U. than the other 2 extracts.

A comparison of the follicular fluid extract and the residual ovarian extract is plotted in figure 4. Here it can be seen that 2 curves show a marked similarity in the uterine response, the follicular fluid extracts showing greater uterine response than the residual ovarian extract. Figure 5 is a summary of all, shown by way of comparison.

DISCUSSION

In figures 1 and 2 it is clearly seen that the uterine response to the extracts of the follicular fluid and of the residual ovary resembles more nearly that of estrone than estradiol. In figure 3 the curves show fairly close resemblance to each other. Doisy believes that estradiol is the principal estrogenic compound in the follicular fluid (25), but the uterine response is far from similar. His belief that the major part of the estrogenic compound is one and the same substance is borne out by the curves in figure 3. Doisy states (26) that he has prepared estrone in surprisingly large amounts from the ovarian extract.

There are several angles from which one may view these results. First, is the comparison of the extracts from non-crystalline material with solutions of crystalline estradiol and estrone. It is generally thought that such comparisons are not true ones. But estrogenically there should be a comparison. Second, from figure 3, and since both the follicular fluid extract and that of the individual ovary are non-crystalline, one could conclude that they con-

tain the same estrogenic compound. Third, these 2 extracts more nearly resemble estrone than estradiol in uterine response. Fourth, if they are identical and if they are estradiol, then some inhibiting substance is present that prevents these extracts from showing the hypertrophy of the uterus exhibited by estradiol. The residual ovarian extract failed to disturb the estrous cycle of both immature and mature normal female rats (12). There may be more inhibiting substances in ovarian tissue than there are in follicular fluid which would account for the differences in their uterine response, or the follicular fluid extract may be principally estradiol, and that from the residual ovaries may be largely estrone. There has recently been reported a much less active estrogenic compound known as β-estradiol. The noncrystalline extracts from follicular fluid and from residual ovarian tissue may contain a significant amount of β-estradiol, which would account for the low uterine response from these 2 sources.

The action may be through the ovary, or its effect counter-balanced by a secretion from the ovary, as this mechanism is not functional in the castrates. Progesterone is known to be antagonistic to estrone. But in the extracts, only 1/50 of a R.U. of progesterone is in the extract per U of estrogenic substance (5). However, the non-response of the normal female to the estrogenic substance may be inherent in the animal itself, or the inhibiting substance present may act through the ovary itself, or may be in the functioning ovary.

The author wishes to express his appreciation to two of his students, Mr. Dan Rickert and Mr. J. Leon Sealey, for help on various phases of this problem.

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CLINICAL AND LABORATORY NOTES

THE USE OF COLCHICINE IN THE ASSAY OF ANDROGENS

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THE EFFECT of androgens on mitotic activity in the seminal vesicle of the castrate rat or mouse was first used by Loewe and Voss[1] for the assay of these substances. The test was considered positive if mitoses were found in the epithelium of the seminal vesicles of the castrate rodent 48 hours after injection of the substance to be tested. This test has not been widely accepted as the finding of mitosis is not uniform. Even in rapidly growing organs the incidence of mitosis is not high. In an animal treated even with large doses of androgens mitoses may be found in some sections of the seminal vesicles but not in others.

Through the work of Dustin[2] colchicine has become a valuable help in studying mitotic activity. This alkaloid arrests mitosis in metaphase. Mitotic figures in cells which divide during the period in which colchicine is active are accumulated. The use of this substance furnishes an excellent method of gauging growth activity due to hormonal action as has been shown first by Allen, Gardner and Smith[3] in studying the effect of estrogens upon the female genital tissues.

In 1937 several groups of workers independently described the use of colchicine in the study of the growth response to androgens of the male accessory sex organs.[4,5,6,7] The onset and duration of this reaction were studied by Tislowitz.[8]

In continuing our work we have found colchicine of considerable practical value for the assay of androgens in urine and tissue extracts for clinical purposes. The following procedure has given the best results.

Extraction. In tissue assays organs are extracted by the method of Moore.[9] The fresh organ is ground to pulp, then extracted with 4 volumes of 95% alcohol at room temperature. The active material is taken up from the concentrated alcoholic extract in benzene. This is evaporated in vacuo, leaving a residue that is taken up in acetone and permitted to stand in the icebox for some hours. After evaporation of the acetone the solids are taken up in 2.0 cc. of olive oil.

For the assay of urine 1000 cc. of urine are acidified with concentrated HCl to a

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[1] LOEWE, S., AND H. E. VOSS: *Deutsch. med. Wchnschr.* 56: 1256. 1930.

[2] DUSTIN, A.-P.: *Bull. acad. med. belg.* 14: 487. 1934.

[3] ALLEN, E., G. M. SMITH AND W. U. GARDNER: *Endocrinology* 21: 412. 1937.

[4] MARTINS, T.: *Compt. rend. Soc. de Biol.* 126: 131. 1937.

[5] BASTENIE, P., AND S. SYLBERSAC: *Compt. rend. Soc. de Biol.* 126: 891. 1937.

[6] MANUS, M. B. C.: *Acta brev. neerland.* 7: 175. 1937.

[7] FLEISCHMANN, W., AND S. KANN: *Akad. Anz. Akad. d. Wissensch. Wien* #21. 1937; *Biochem. Ztschr.* 296: 373. 1938.

[8] TISLOWITZ, R.: *Abstr. XVI Internat. Physiol Congr. Zurich.* 1938; *Endocrinology* 25: 749. 1939.

[9] MOORE, C., in Glandular Physiology and Therapy, American Medical Assoc., Chicago, 1935.

pH below 1, boiled for 15 minutes and after cooling extracted with benzene for 24 hours in a continuous extractor. After evaporation of the whole amount or an aliquot, the residue is taken up in 2.0 cc. of olive oil.

Bioassay. The olive oil solution is injected in two doses of 1.0 cc. each into castrate rats. It is important to use for assay only rats which have been castrated 4 weeks previously. The body weight at the time of castration should be about 50 gm. If rats are used shortly after castration, mitosis may occur spontaneously or possibly may be induced by colchicine. However, colchicine induces mitosis only in cells in which it is imminent and never in cells of an atrophied organ such as the accessory sexual organs of a castrate animal. The second injection of the material to be tested is given 18 hours after the first. Twenty-four hours after the second injection the animal is injected subcutaneously with colchicine. The dose is 0.1 cc. of a 1:1000 aqueous solution of colchicine per 100 gm. of body weight. Larger amounts may be fatal. The colchicine solution is prepared immediately before use. To facilitate solution 25 mg. of colchicine (Mallinkrodt) are dissolved in 2 cc. of 80% alcohol in a 25 cc. volumetric flask, which is then filled up to the mark with water. The animals are killed 8 hours after the injection of colchicine. The seminal vesicles are removed as quickly as possible and fixed in Bouin's fluid.

Histology. It is advisable to fix a small section of the small intestines at the same time as the seminal vesicles. The sections are stained either with hematoxylin-eosin or with iron-hematoxylin. The sections of the intestine show numerous arrested mitoses in the epithelium. This is a proof that the colchicine dose has been effective. In the seminal vesicles mitoses appear in appreciable number only if a sufficient amount of androgen is present. The test is to be considered positive if 15 or more mitoses can be discovered in one visual field with a magnification of about 200 diameters. The cells, in which mitosis was arrested through the action of colchicine are easily recognized by the dense, usually pyknotic, chromatic nuclear material and the apparent increase of clear cytoplasm.

In testing pure androgens dissolved in sesame oil the following results have been obtained with this method. The amounts indicate the total of the 2 doses injected.

Androsterone	0.1 mg.	negative
Androsterone	0.2 mg.	negative
Androsterone	0.5 mg.	positive
Testosterone propionate	0.02 mg.	negative
Testosterone propionate	0.05 mg.	positive
Testosterone propionate	0.1 mg.	positive

The end point of this reaction is fairly sharp. It is easier to interpret the onset of growth response in the seminal vesicles by the finding of groups of cells with arrested mitosis than by any other cytological criterion. As the effect of 0.1 mg. androsterone is the accepted standard for one unit of androgen, a positive reading by the colchicine technic indicates the presence of at least five international units. It must be remembered that this test, like all tests on the male accessory of the castrate, is not strictly specific for androgens. Estrogens also can induce mitosis in the seminal vesicle of the castrate rodent.[7,8] However, the effect of androgens in the 50-hour experiment is much greater than that of a comparable amount of estrogens.

In the capon comb test it has been shown[10] that testosterone propionate is 5 times as potent as androsterone, 0.02 mg. of testosterone propionate producing the same effect as 0.1 mg. of androsterone. By our technic testosterone propionate is 10 times

[10] GOLDBERG, M. W.: *Ergebn. d. Vitamin- und Hormonforschung* 1: 371. 1938.

as potent as androsterone since a positive test is obtained with 0.05 mg. testosterone propionate and with 0.5 mg. androsterone. This is in accordance with the established fact that the accessory sex organs of the rodent are relatively more sensitive to testosterone and its esters than the comb of the capon or baby chick.

Friedgood and Whidden[11] have recently pointed out the difficulties of an exact assay of total urinary androgen excretion by chemical or biological methods. However, the colchicine method described above can be used for approximate determination of androgens.

Estimation of the androgenic activity of a known amount of urine can be made by determining the minimum amount of extract which gives a positive reaction. If, for instance, an aliquot corresponding to 500 cc. of urine gives a positive reaction, the aliquot corresponding to 250 cc. a negative reaction, the androgen content of the urine can be estimated at $1000 \times 5/500 = 10$ I.U. per liter.

Summary. By arresting mitosis with colchicine, a clear cut histological picture of cellular stimulation can be obtained. By this method the stimulating effect of androgens upon the seminal vesicle of the castrate rat is sharply defined. A positive test (the finding of numerous mitoses) is given by 0.5 mg. of androsterone (5 I.U. androgen) and by 0.05 mg. testosterone propionate. With smaller doses only occasional mitoses are found. The method can be used for estimating the androgenic activity of tissue or urine extracts.

[11] Friedgood, H. B., and H. L. Whidden: *New England J. Med.* 220: 736. 1939.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

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BOOK REVIEWS

Estudio Anatómico de 135 Tumores de la Hipófisis y del Tracto Hipofisario, con algunas consideraciones clínicas, histogénicas y fisiopatológicas. I. Costero and H. Berdet. Monografia Num. 1, de la Sociedad Medica del Hospital General, Mexico, D. F. May, 1939.

A study is presented of 135 pituitary tumors studied histologically and classified as to origins. It appeared that the epithelium of the primitive hypophyseal vesicle may develop in two different directions leading to completely differentiated adult structures, squamous cornified epithelium or adamanatinoid epithelium, both of unquestionable oral (prostomic, ectodermal) origin, and ciliated epithelium with mucin, tubular glands and even tonsil-like lymphatic formations. The latter are from rhinopharyngial (endodermal?) origin. In the anterior lobe of the hypophysis cerebri there are 4 types of cells which are capable of producing individualized tumors: the main cells, the acidophilic cells, the basophilic cells and certain large elements, probably related to the acidophilic cells. The chromophobe adenomas derive from cylindrical elements which line the hypophyseal vesicle and are the only glandular cells of the adeno-hypophysis capable of producing malignant tumors. The main cells of pituitary adenomas are often transformed into typical acidophilic cells. An intermediate type of cell is very abundant in adenoepitheliomas. The basophilic cells appear in tumors independently of the other glandular cells. None of the tumors of the hypophysis were found derived from 'pituicytes.' The 'pituicytes' are considered as common neuroglia cells. In the adenomas of the pituitary body the chromophobe cells do not produce hormones, but a large quantity of colloid. Acidophilic granulations in the cytoplasma of the tumor cells are strictly in relation to acromegalic syndromes, the intensity of which is proportional to the quantity of acidophilic matter in the tumor. The rôle of basophilic granulations in Cushing's syndrome is considered insufficiently established. The work is properly illustrated with photomicrographs and the format is pleasing.

Endocrinologia. Glandulas de Secreción Interna. P. Engel, in collaboration with Benjamin Muñoz Giraldo. Editorial Rozas, Bogota, 1939. Pp. 214.

In this little book the author sets forth in an engaging way a substantial proportion of the more significant aspects of endocrinology as known today. His citations show a catholic familiarity with the international literature and from this he has selected judiciously. Those who are able to read Spanish will find the book a valuable epitome.

DIABETES INSIPIDUS AND THE NEURO-HORMONAL CONTROL OF WATER BALANCE: A CONTRIBUTION TO THE STRUCTURE AND FUNCTION OF THE HYPOTHALAMICO-HYPOPHYSEAL SYSTEM. Charles Fisher, W. R. Ingram and S. W. Ranson. Edwards Brothers, Inc. Ann Arbor, Michigan, 1938. Pp. 212.

"Diabetes insipidus is essentially a hypophyseal-deficiency syndrome caused by a diminution or total absence of the antidiuretic hormone of the neural lobe of the hypophysis when the latter is extirpated or becomes atrophic secondary to interruption of the supraopticohypophyseal tracts." Thus is given the answer to a complex problem that has engaged investigators for many years. The author's final solution of the problem was reached by studies of the hypothalamus on several hundred cats and a considerable number of monkeys. Their series of 85 cats and 2 monkeys in which diabetes insipidus was successfully produced is estimated as probably equalling the combined totals of previous investigators. Through use of the Horsely-Clarke stereotaxic instrument it was possible finally to circumvent the various difficulties that had long delayed the final solution.

Not only are the authors' own results set forth in instructive detail but the monograph also includes a searching historical study of the whole problem of the control of water exchange in the body. The thoroughness of the undertaking is suggested by the bibliography of some 350 titles that are cited. The relative parts played by the hypophysis and the hypothalamus are discussed at length. In three penultimate chapters the relation of the thyroid and of the sex glands to water metabolism is discussed—largely on a basis of the authors'. own work. The book closes with a chapter on the site of formation of the posterior-lobe hormones.

The study might well serve as a model for the conducting and the reporting of a major endocrine research. Despite the wealth of technical details that are presented the clarity of style renders the reading easy and pleasurable. A well-selected series of half-tone and line illustrations add materially to comprehensibility and attractiveness. The use of the photolithograph process rendered possible publication of the book at a materially lower price than would have otherwise been possible. The authors may be commended for supplying so attractive a bait to induce scientific readers to become accustomed to this style of producing monographs.

Despite the proverbial danger of prophesying, the prediction is offered that this monograph will take a place among the classics of endocrinology and prove to be one indispensable to students of either the hypothalamus or of water metabolism.

TACTIQUE OPÉRATOIRE DES GLANDES ENDOCRINES. G. Jeanneney and P. Foucault. G. Doin & Co., Paris, 1938. Pp. 186.

The contents of the book are clearly indicated in the title. Special directions are given for the surgical approach to the various endocrine structures and for operative details. Numerous illustrations add to clarity. Directions for gonad grafting are also included. The book is well printed and readable.

MANUAL DE LAS ENFERMEDADES ENDOCRINAS Y DEL METABOLISMO. G. Marañon. Librería Hachette S. A., Buenos Aires, 1938.

Although Professor Marañon has previously treated monographically the whole endocrine field as well as special parts of it, this book has been written so nearly de novo as essentially to constitute a new work.

Although it follows what has become largely the conventional pattern of textbooks in endocrinology it acquires uniqueness from the wide personal experience of the author both in the laboratory and in the clinic.

After a series of introductory chapters on such topics as interrelations of the hormonal and nervous systems, chemistry of the hormones and anatomical changes in relation to functions, the various glands are taken up seriatim. Emphasis is laid upon clinical considerations but a special chapter is devoted to laboratory tests. A final section deals with diseases of metabolism. The work is illustrated with a variety of well-selected photographs.

THE 1938 YEAR BOOK OF NEUROLOGY, PSYCHIATRY AND ENDOCRINOLOGY. Edited by Hans H. Reese, Harry A. Paskind and Elmer L. Sevringhaus. The Year Book Publishers, Chicago, 1938. Pp. 776.

The endocrine section in this increasingly popular work has again been written by Sevringhaus. It is noteworthy both for the wide coverage of the world's important endocrine literature and for the skill with which selections have been made and expounded.

While the major part of the material consists of reiteration of the data of the various articles abstracted the author has skilfully placed the material in a general endocrine frame of reference so as to bring out not only technical content but also broader relationships. Numerous critical comments, largely based on the author's own extensive experience add materially to the value of the work.

To any one who desires a readable and well-organized bird's-eye view of recent major accomplishments in endocrinology the book is highly commended.

MAJOR ENDOCRINE DISORDERS. S. Levy Simpson. John Bale Medical Publications, Ltd., London, 1938. Pp. 184.

The appearance of this book suggests an endocrine primer of the type that secures simplicity by omitting most of the endocrinology and diluting the remainder. Actually, it is a scholarly condensation that includes in less than 200 small pages a surprisingly large proportion of the present day endocrinology that is clinically significant.

The work follows the standard pattern of a good textbook of medicine, describing the diseases that arise in malfunctioning of the various glands and discussing their differential diagnosis, pathology and treatment. Interspersed historical bits add to the interest of the book. Its up-to-the-minuteness is indicated by a brief section on 'diabetes tenuifluus,' a condition ascribable to over-secretion of pituitary antidiuretic hormone.

Among numerous attractive sections, those on obesity and the adrenogenital anomalies are of noteworthy excellence.

The text, though succinct, is clear and readable. A considerable number of well-selected photographs add graphicness to the verbal descriptions. Altogether the book is practical in the best sense of that much-abused word.

STUDIES FROM THE CENTER FOR RESEARCH IN CHILD HEALTH AND DEVELOPMENT, SCHOOL OF PUBLIC HEALTH HARVARD UNIVERSITY. I. THE CENTER, THE GROUP UNDER OBSERVATION, SOURCES OF INFORMATION, AND STUDIES IN PROGRESS. Harold C. Stuart and Staff, Society for Research in Child Development, National Research Council, Volume IV, No. 1 (Serial No. 20), 1939. Pp. 261.

This is an orienting report on the first 7 years' work of the Center for Research in Child Health and Development at the Harvard School of Public Health. It deals largely with the empirical experiences in methodology and appraisals of values of the various methods. The technic of individual and of family-record analysis is illustrated in detailed case histories of two sisters. In addition, a final section of the report serves to indicate the variety of information now available in the records of the Center. All those who are technically interested in systematic studies of child development will want to familiarize themselves with this valuable material.

ADVANCE ABSTRACTS

BASSLER, A. Three cases of masked hypothyroidism having abdominal symptoms. *To appear in this Journal.*

In these 3 cases suggestions of a partial type of myxedema may be deducted from the history and physical findings. They present low blood pressure, leukopenia and low B.M.R., all of which are determined by laboratory procedure. These cases, because they come under observation with abdominal complaints, may escape proper diagnosis. They are dramatically benefited by thyroid therapy. In them there seems to be a diversity of complaints and physical findings. The significance of the low thyroid condition is liable to be lost despite the fact that it is the main cause of the illness. Why there is abdominal pain

and tenderness in this condition is not understood. It may be neurogenic in origin; it certainly is not due to colonic stasis or constipation.

EIDELSBERG, J., AND E. A. ORNSTEIN. Observations on the continued use of male sex hormone over long periods of time. *To appear in this Journal.*

The report attempts to answer the questions arising as to results of treatment over long periods of time in hypogonadism (of various types) with testosterone propionate (oreton)—4 cases treated, for about 16 to 18 months. The 4 cases detailed are: (1) A male, age 28 years, with a pluriglandular dyscrasia, the predominant picture being hypogonadism, atypical eunuchoidism and gynecomastia; treated with 50 to 60 mg. oreton weekly; the response described; the total amount of testosterone propionate used thus far—2800 mg.; (2) A male, age 21 years, diagnosed as post-castration, typical eunuchism, treated with 50 to 60 mg. oreton weekly; the results described, and the total amount of testosterone propionate used thus far being 3250 mg.; (3) A male, age 21 years, diagnosed as a modified Lorain-Levi hypopituitarism, treated with 50 to 60 mg. oreton weekly, and the results detailed; the total amount of testosterone propionate to date being more than 3400 mg.; (4) A male, 28 years of age, typical Lorain-Levi Infantilism, given 50 to 60 mg. oreton weekly, and the results described, having used 3500 mg. testosterone propionate to date. Some of the effects are changes in the physical characteristics, changes in configuration, growth of axillary, supra-pubic and body hair, slight growth of facial hair, change in voice, growth of Pomum Adami, growth of genitalia, growth of scrotum, appearance and increase of ejaculations, appearance and increase of libido and erection, desirable and agreeable psychic and mental changes, gain in weight. Some of the points made, include the teaching of self-administration (each patient regarding himself deficient in testosterone, as the diabetic is deficient in insulin); it is replacement therapy; omission of treatment for two or more weeks brings regressions; the average maintenance dose is 50 to 60 mg.; continued therapy has brought no untoward symptoms; combined therapy may be necessary for optimum benefits; and that the uncontrolled sale of testosterone propionate should be replaced by a controlled sale on prescription (as with narcotics).

EMERY, F. E., T. J. BUGELSKI AND E. L. SCHWABE. The response of the ovaries to gonadotropic extracts in splenectomized rats. *To appear in this Journal.*

That the spleen may be of considerable importance in the formation of antihormones has been previously reported. The present study deals with some commercial extracts commonly in use at the present time. A total of 83 young rats splenectomized at approximately 30 days of age were injected for a period of 30 days with antuitrin-S, A.P.L. and pituitary gonadotropic extract. The weights of the ovaries and uterus at the end of treatment showed that these organs were not significantly different from those of control rats similarly injected. The ovaries of some of these rats in both the splenectomized and control groups were apparently not increased in weight beyond the mean ovarian weight of normal control rats. The percentage of time in estrus was below normal in all of the injected groups.

FRIEDGOOD, H. B., AND H. L. WHIDDEN. Determination crystalline androsterone with the Evelyn photoelectric colorimeter. *To appear in this Journal.*

A modification of the Zimmermann procedure for color development has been applied to the assay of crystalline androsterone, and the Evelyn photoelectric colorimeter has been calibrated for the technic in terms of androsterone. The greatest advantage gained by the use of this colorimeter is the elimination of the subjective factor inherent in visual comparative measurements. The data obtained indicate a high degree of reliability and reproducibility of results in the assay of crystalline androsterone.

GILMAN, J. Experimental studies on the menstrual cycle of the baboon (Papio porcarius). VI. The effect of progesterone upon the first part of the cycle in normal female baboons. *To appear in this Journal.*

The normal adult female baboon, when given progesterone in the first or turgescent part of the cycle, exhibits specific perineal reactions which may or may not be accompanied by bleeding at the end of 4 to 7 days after progesterone administration. The perineal reaction consists of a sudden onset of deturgescence when the sex skin may become completely absorbed. The minimum quantity of progesterone necessary to produce this effect is 3 mg. Bleeding occurs if a single dose of 20 mg. progesterone is used and is precipitated on the 5th day after the injection when the perineal swelling has reached its lowest ebb. Doses smaller than 20 mg. produce perineal deturgescence unaccompanied by bleeding. If 2 or 3 injections are given at 3 or 4-day intervals then a total of 15 mg. of progesterone is adequate to produce bleeding. Progesterone withdrawal is capable of precipitating bleeding and in so doing the normal cycle of a baboon can be shortened to one-third its normal length. Where bleeding is not produced by the single administration of progesterone the cycle is prolonged by 3 to 7 days as a rule. The administration of appreciably large quantities of progesterone (10 mg.) early or late in the first part of the cycle has no effect on the bleeding. A minimal amount of progesterone is necessary to produce perineal deturgescence without bleeding and perineal deturgescence with bleeding. Progesterone acts in the presence of large quantities

of estrone and the action of progesterone is not fully explained by a quantitative inactivation of estrone. In the baboon the uterus and perineum, after they have been sensitized to estrone, have a greater affinity for progesterone than for estrone and progesterone is active in the presence of large quantities of estrone. Two reactions of the baboon, perineal deturgescence without bleeding and perineal deturgescence with bleeding permit an easy and accurate assay of progesterone uncomplicated by operative procedures.

KENYON, A. T., K. KNOWLTON, I. SANDIFORD, F. C. KOCH AND G. LOTWIN. A comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. *To appear in this Journal.*

In confirmation of our previous studies, intramuscular injections of 25 mg. of testosterone propionate, daily or twice daily, in a eunuchoid, produced reduction in the urinary excretion of N, Na, K and Cl, together with a gain in weight, due largely to water held in association with the salts and protein retained. A considerable reduction in the urinary excretion of inorganic P accompanied that of the other constituents. During recovery Na, Cl and water were rapidly lost from the body, inorganic P and K less rapidly and less completely, while N stored was retained in great part for weeks. Two normal young men responded like the eunuchoids, except that the 0.03 gm. of N retained per kg. per day, during the period of maximal effect, was half that of the eunuchoids. One normal young woman responded essentially as the eunuchoids and the normal men. The second normal young woman showed a definite reduction in the urinary excretion of inorganic P but otherwise distinctly less evidence of a testosterone propionate effect. The reasons for the differences between these women are not clear. The concentrations of urea and N.P.N. of the blood were definitely reduced by such treatment, while those of hemoglobin, plasma proteins, and serum Na, K and Cl were unaffected. Inorganic P concentration in the serum was doubtfully reduced. The reductions in urinary electrolyte excretion, studied in the eunuchoid, were unaccompanied by important changes in urinary ammonia, pH, bicarbonate or concentration of titratable acid. Creatine excretion, in the eunuchoid, intensified by ingestion of creatine, was substantially reduced by the androgen and increased during a recovery. Percutaneous administration of 25 and 50 mg. daily of testosterone itself in an ointment, in the eunuchoid, produced diminution in the urinary excretion of N. The B.M.R., fasting R.Q., pulse rate and blood pressure of the normal subjects were not conspicuously affected by testosterone propionate. The B.M.R. of the eunuchoid was probably slightly increased; the pulse rate and blood pressure were unaffected. The 102.7 to 252.2 gm. of protein estimated as retained by these subjects is not accounted for by increased in the bulk of genital tissues and represents deposit of new material elsewhere in the body. This together with the reduction in the urinary excretion of inorganic P, K and creatine points to a somatototropic influence of androgens, and provides a clue to the explanation of unusual body growth accompanying precocious puberty.

KOCHAKIAN, C. D. The tolerance of male and female mice respectively to estrogens and androgens. *To appear in this Journal.*

Each sex of the Little dba mice was better able to tolerate the 'sex hormone' of the opposite sex. Testosterone propionate in daily doses of 0.4 mg. and 0.20 mg. was not fatal but caused a decrease in gain of body weight in the males. Estradiol dipropionate in doses of 0.25 and 0.10 mg. per day was fatal but more so in the females than the males. Testosterone propionate added to the latter in 4 times its quantity was able to nullify in part but not completely the fatally toxic properties of the estrogen. Testosterone propionate caused a definite and striking loss of hair in the male mice and only a slight effect in females. The controls showed no effect.

LOONEY, J. M. The treatment of pituitary dwarfism with growth hormone. *To appear in this Journal.*

A case report is given of a girl of 13, showing pituitary dwarfism. The child was found to weigh 28.8 kg. and measure 138.7 cm. in height as compared with the minimum values for her age of 35.5 kg. and 146.5 cm. She was treated for 2 years with pituitary extracts containing growth factor (Armour), with thyroid, vitamins and dicalcium phosphate. During this period she gained 10 cm. in height and 11.8 kg. in weight, which exceeded the normal expectations for a girl of this height by 3.5 cm. in height and 3.8 kg. in weight. The enuresis and failure in school for which the child was referred to the Child Guidance Clinic was markedly improved at the same time.

PASCHKIS, K. E., AND A. SCHWONER. The output of protein metabolism hormone of the pituitary anterior lobe. *To appear in this Journal.*

Protein feeding is followed by the output of a factor in the urine which, injected into rats produces a decrease of blood urea N and of blood amino N. This factor is not present in the urine after a protein-free carbohydrate-fat diet. This reaction to injection of urine following protein intake (decrease of N.P.N. constituents in the rat) is the same as is obtained by injecting alkaline extracts of the pituitary. It is highly probable that the factor traced in the urine after protein feeding is of pituitary origin. As has been previously shown a factor of pituitary origin also prevents rise of blood amino N after a protein meal

given 3 hours after a similar previous protein meal. Protein feeeding is the adequate stimulus for the output of this hormone. Stimulation of the output of hormone by administering the substance on which the hormone specifically acts is not peculiar to protein metabolism. The same occurs in carbohydrate, fat and calcium metabolism and we may therefore assume that this mechanism represents a general physiological rule.

SANDBURG, M., D. PERLA AND O. M. HOLLY The metabolsim of nitrogen, sulfur and glutathione in thymectomized albino rats. *To appear in this Journal.*

The influence of thymectomy on the metabolism of N and S including the S-bearing tripeptide glutathione was studied in male and female albino rats. Urinary N excretion increases progressively, fecal excretion remains unchanged. Exogenous as well as endogenous S metabolism is disturbed. Total urinary S as well as neutral S excretion increases. Fecal S, inorganic and ethereal sulfate excretion remains unchanged. Total glutathione in the blood of thymectomized rats rises slightly, but begins to return to normal 6 weeks after operation. A drop in reduced glutathione develops more slowly, but persists longer. The main disturbance is encountered in oxidized glutathione, which rises considerably after operation, and 2 months later still shows high values. But eventually adjustment takes place and 18 weeks after thymectomy all glutathione values have returned to normal. The interrelationship between the thymus and other glands of internal secretion has been discussed.

STURGIS, S. H., AND F. ALBRIGHT. The mechanism of estrin therapy in the relief of dysmenorrhea. *To appear in this Journal.*

In a patient with essential dysmenorrhea, a course of estrin therapy, if administered in accordance with certain specifications, will result in the absence of dysmenorrhea during the following but not during subsequent menses. To produce this result, the course of estrin therapy should be started about the sixth day of the cycle and should be of the order of magnitude of 1.5 mg. of estradiol benzoate intramuscularly every 3 days for 6 injections. Such therapy prevents the formation of a secretory endometrium as demonstrated by endometrial biopsy presumably due to an inhibitory action of estrin on gonadotropic hormones and hence on ovulation. From this and other collateral clinical evidence it is concluded that essential dysmenorrhea cannot occur in the absence of a secretory endometrium.

TWORT, J. M., AND R. LYTH. Correlation of pituitary and spleen in experimental and control mice. *To appear in this Journal.*

Data relating to the weight of spleens and approximate superficial area of pituitary gland in over 4,000 mice, and data relating to the pituitary dry weight of over 2,500 have been analyzed in order to find out whether the weight of the spleen per se influenced the size of weight of the pituitary gland. The mice were grouped according to whether they were untreated controls or experimental and the experimental were further grouped according to whether they bore benign or malignant tumors or were tumorless. The groups were sub-grouped according to sex. Each group was divided into classes according to total body weight, (15 to 41 gm). and the mean value for spleen and pituitary for each class determined. Analysis of our figures revealed that there was a slight positive correlation in all the groups except possibly in the malignant one. No obvious differences as regards sex were noticed in this connection. When mice with large spleens only were considered in the various classes a definite positive correlation was found between the two glands in all groups. There was some evidence of a negative correlation between spleen and pituitary in tumor-bearing mice which had very small spleens but unfortunately the number here were too small to draw definite conclusions. Small spleens of tumorless mice and controls, however, tended to give positive correlation with their pituitaries.

UOTILA, U. U. The effect of estrin on the anterior pituitary of male rats after pituitary stalk section. *To appear in this Journal.*

Large doses of estrin injected into male rats act on the anterior pituitary without the mediation or any modifying effect of the pituitary stalk. This is evidenced by the following facts: (1) the anterior pituitary undergoes hypertrophy and shows the same cytological changes in rats which have received estrin whether the stalk be intact or severed; (2) estrin suppresses the secretion of gonadotropic hormones in stalk-cut and intact male rats to an equal extent, as shown by the appearance of a marked atrophy of the testicles and seminal vesicles in both groups. The adrenals hypertrophy equally after estrin treatment in intact and stalk-sectioned animals, indicating that estrin also affects the corticotropic function of the anterior hypophysis without any mediation or modification of the pituitary stalk. It is concluded that estrin acts directly on the anterior hypophysis, and that this action is a part of the humoral control of the basic secretory rhythm of the gland.

WHITESIDE, B. The effect of thyrotropic hormone upon pregnant rabbits. *To appear in this Journal.*

The fact that the administration of thyrotropic hormone causes an interruption of pregnancy was proved by Glaubach. The present paper is a histological study of her material which consisted of preg-

nant rabbits injected with thyrotropic hormone. The first group of animals were injected for a period of about 14 days, the second group for 5 days. In every case there was an interruption of pregnancy, either by abortion or by the death of the fetuses. A histological examination was made of the fetuses and of the thyroid and ovary of the maternal animals. The majority of the fetuses of group I were entirely degenerated. The fetuses of group II showed normal organs. The thyroid of the maternal animals of both groups showed a hyperplasia greater than the normal hyperactivity in pregnancy. There was a small percentage of cases of involution of the thyroid. The ovary of the maternal animals in group I showed signs of degeneration similar to those obtained with thyroxin injections. In group II the ovary appeared normal. The histological results are interpreted as support for the theory that the thyrotropic hormone interrupts pregnancy by stimulating the thyroid gland to excessive activity.

ADRENALS

Ausländer, E. M. Immediate and late results of transplantation of adrenal cortex in fourteen cases of Addison's disease. *Novy khir. arkhiv.* 42: 375. 1939.

The author reports the immediate and late results of 20 transplantations in 14 cases of Addison's disease. The adrenal was removed immediately on the killing of the animal at the abattoir and the cortex was dissected from the medulla in the operating room. Fragments of the cortex were implanted into the subcutaneous tissue of the abdomen. The immediate results were excellent in all of the 14 cases. The patients exhibited an increase in weight, in the blood pressure, in the blood cholestrol and in the blood sugar, as well as an increase in the muscle power. Nausea, vomiting, diarrhea and low gastric acidity disappeared. The general health was improved to such a degree that patients who were invalids for one to two years were able to resume their occupations. More or less permanent results were obtained in the mild cases and in the cases of medium severity. The longest period of observation was one year and 8 months. In a few cases the improvement was progressive. In the severe cases the improvement lasted from 5 to 6 months, after which there was a gradual return of the symptoms, necessitating in from 9 to 12 months a second transplantation. These second transplantations gave equally good results. The author concludes that the transplanting of the adrenal cortex is superior to any of the other therapeutic measures in Addison's disease.—From *J. A. M. A.* (Abbreviated).

Benetato, G., N. Munteanu and R. Oprean. Action of hormones on the colloidal state of tissue proteins (Action de certaines hormones sur l'état colloïdal des protéines tissulaires). *Compt. rend. Soc. de biol.* 129: 780. 1938.

Experiments on senile rats were made to determine the effects of hormones on the solubility of tissue proteins. Their solubility was increased by adrenal cortical hormone and especially by cortical hormone combined with male hormone, but not appreciably by growth hormone. It was stated that these results suggest a use of hormonal therapy in correcting morphological and functional disorganization of cells.—E. Cutuly.

Britton, S. W., and R. F. Kline. Augmentation of activity in the sloth by adrenal extract, emotion and other conditions. *Am. J. Physiol.* 126: 127. 1939.

In several hundred tests the sloth was observed to progress on the average at the rate 1 mile in 3 to 4 hours. Under excitation its speed was increased 25 to 50%, in some cases more than 100%. Both two-toed and three-toed species were used; the latter were the slower animals. Emotional excitement and rise in body temperature were very effective stimulants to increased rate of movement. Cortico-adrenal extract, adrenalin and prostigmin also markedly increased bodily activity. The beneficial influence of the cortical hormone lasted as long as 12 hr. Thyroxin, thyroid extract, pituitary extract, benzedrine and strychnine sulphates were tried and found ineffective.—From the Author's Summary.

Cammer, L., and F. R. Griffith, Jr. Adrenaline and the metabolism of peripheral tissues. *Am. J. Physiol.* 125: 699. 1939.

In 48 cats under chloralose anesthesia arterial and venous blood of one hind leg was sampled and the rate of blood flow studied before and after intra-arterial injections of salt solutions and adrenaline. Adrenaline in effective concentrations appeared to decrease the blood flow and O_2 consumption. The CO_2 output was affected in a complex manner but the production of CO_2 was apparently reduced. The well known calorigenic effect of adrenaline on the intact animal must take place in some region other than the peripheral tissues.—G. C. Ring.

Corey, E. L., and S. W. Britton. Hypophyseal and adrenal interrelationships and carbohydrate metabolism. *Am. J. Physiol.* 126: 148. 1939.

Cortico-adrenal extracts free from adrenalin and choline were found to produce a prolonged elevation of blood sugar. Fasting hypophysectomized rats show subnormal amounts of blood sugar and liver glycogen. These are increased to normal (or supernormal) levels after injections of cortico-adrenal extracts.

Muscle glycogen is affected in a similar but less striking manner by the above procedures. These results give evidence that the hypophysectomized animal has cortico-adrenal insufficiency.—G. C. Ring.

COREY, E. L., H. SILVETTE AND S. W. BRITTON. Hypophyseal and adrenal influence on renal function in the rat. *Am. J. Physiol.* 125: 644. 1939.

Hypophysectomy in the rat was followed by polyuria and polydipsia which subsided in a few days. No such effects were observed if the adrenals were removed at the same time. After hypophysectomy, adrenalectomy or hypophyso-adrenalectomy there was a reduction of urinary chloride. However, the latter was concentrated to high levels a few days later. Injection of post-pituitary solution into hypophysectomized or adrenalectomized rats not only prevented the reduction of urinary chloride but raised it two or three times above normal. Adrenal extract prevented hypochloria in adrenalectomized animals but was ineffective in hypophysectomized animals. The results indicate that there is direct involvement of the post-pituitary mechanism in the regulation of the salt and fluid balance of the body and that the adrenal has an indirect action on salt metabolism. It is suggested that the posterior pituitary and the adrenal cortex are antagonistic in their action on fluid metabolism.—From the Author's Summary.

DOBROVOLSKAÏA-ZAVADSKAÏA, N., AND P. ZÉPHIROFF. The adrenal in strains of mice with different susceptibilities to cancer (La surrénale dans les lignées de souris a potentialité cancérigene différente). *Compt. rend. Soc. de biol.* 128: 971. 1938.

A series of mice of a strain with a high incidence of spontaneous mammary cancer was observed to have adrenals in which the juxtamedullary zone revealed large areas of lipoidal degeneration. This cortical degeneration was not seen in strains of mice not subject to mammary carcinoma.—E. Cutuly.

DRAKE, M. E., J. F. RENSHAW, F. S. MODERN AND C. H. THIENES. The smooth muscle actions of epinephrine substitutes VII. Responses of denervated smooth muscles of iris and intestine to epinephrine, ephedrine, amphetamine (benzedrine) and cocaine. *J. Pharmacol. & Exper. Therap.* 66: 251. 1939.

The pupils of either the left or right eyes of 5 cats and 4 rabbits were denervated by removal of the superior cervical ganglion. Mydriasis in the normal eyes of both cats and rabbits was produced by the 4 drugs. Denervation of cat's iris prevented mydriasis by ephedrine and did not affect amphetamine mydriasis and increased the epinephrine mydriasis. Denervation of the rabbit iris prevented mydriasis by cocaine or amphetamine, but the effect of ephedrine was not altered and the response to epinephrine was increased. After sympathetic post-ganglionic denervation of loops of the small intestine, the response to ephedrine, amphetamine and cocaine was not altered but the response to epinephrine was increased. The authors believe the action of ephedrine, amphetamine and cocaine is neither sympathicotropic nor musculotropic.—E. L.

FASCIOLO J. C. Arterial hypertension from renal ischemia independent of the adrenals (L'hypertension artérielle par ischémie rénale ne depend pas des surrénales). *Compt. rend. Soc. de biol.* 128: 1130. 1938.

In dogs renal ischemia caused arterial hypertension in the absence of the adrenal glands or of the adrenal medullae.—E. Cutuly.

FUSTINONI, O. Survival in toads after adrenalectomy (Survie des crapauds après destruction des surrénales). *Compt. rend. Soc. de biol.* 128: 1137. 1938.

Adrenalectomy in toads was uniformly fatal. Death usually occurred 2 to 4 days postoperatively; some animals survived as long as 12 days. High temperatures (35° C.) shortened the survival period and low temperatures (5° C.) lengthened it. Injections of cortin, salts and other substances had no effect on the survival period.—E. Cutuly.

FUZII, K. How haemorrhage affects the threshold dose of adrenaline in raising the blood pressure in dogs and rabbits. *Tohoku J. Exper. Med.* 35: 114. 1939.

The author used 26 dogs and 14 rabbits to ascertain the effect of hemorrhage upon the minimal pressor dose of adrenalin. The dogs were anesthetized with morphine but the rabbits were unanesthetized. They were bled from a femoral artery and while the mean arterial pressure was low, the minimal pressor dose of adrenalin was tested. In dogs, the loss of 10% to 30% of the total volume of blood lessened the minimal pressor dose of adrenalin, but the loss of 60% or more enlarged it. In rabbits, where there was a small amount of bleeding, the author found a reduction of the minimal pressor dose and an increase when it was 2% or more of the body weight.—Margaret Baptist.

GERSH, I., AND A. GROLLMAN. The relation of the adrenal cortex to the male reproductive system. *Am. J. Physiol.* 126: 368. 1939.

The development of the secondary reproductive organs of male rats and mice were compared in normal, in castrated and in castrate-adrenalectomized rats and mice maintained on adequate doses of

cortical hormone. The results indicate that the adrenal gland does not exert an androgenic function in the normal animal. Neither the 'juvenile' cells of the rat nor the x-zone of the mouse normally exert an andrgoenic function. The adrenal cortical hormone was found to exert no androgenic effects on the development of the ventral and dorsal prostate glands, of Cowper's gland, or of the seminal vesicles of normal or castrated mice and rats. Extracts of human or pig fetal adrenal glands and of x-zone bearing glands of mice were also found to be devoid of androgenic activity.—Authors' Summary.

GRIFFITH, F. R., JR., J. E. LOCKWOOD AND F. E. EMERY. Adrenaline hyperglycemia: Proportionality with dose. *Am. J. Physiol.* 126: 299. 1939.

The intravenous injection of adrenalin at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700, and 0.01000 mg./kg./min. for 5 min. affected blood sugar level of chloralose anesthetized cats as follows: (*a*) the approximate minimal effective dose was 0.00050 mg./kg./min.; (*b*) the maximum blood sugar level was usually attained in the 10 min. interval following the 5 min. injection period; (*c*) hyperglycemia increased with dose up to 0.004 mg./kg./min., the 2 rates of injection beyond this, i.e., 0.007 and 0.010 mg./kg./min., produced progressively decreasing augmentation of blood sugar level.—From the Author's Summary.

HAMLIN, E., AND M. I. GREGERSEN. The effect of adrenaline, nembutal and sympathectomy on the plasma volume of the cat. *Am. J. Physiol.* 125: 713. 1939.

Continuous intravenous injection of adrenalin (0.0018 to 0.035 mg. per hg. per min.) in unanesthetized normal and in sympathectomized cats caused a small increase in plasma volume. Similar animals under the influence of nembutal showed a decrease in plasma volume after adrenalin injection although the decrease was smaller in sympathectomized animals. Nembutal alone caused an increase in plasma volume. —F. A. H.

HARRISON, H. E., AND D. C. DARROW. Renal function in experimental adrenal insufficiency. *Am. J. Physiol.* 125: 631. 1939.

In adrenalectomized dogs the renal tubules fail to reabsorb Na adequately from the glomerular filtrate and fail to excrete K, phosphate and perhaps other ions at high concentration when these ions are abnormally concentrated in blood plasma. This failure of the tubules explains the change in plasma ions. As a result of the loss of Na and Cl from the body a diminished rate of glomerular filtration occurs which contributes to the final stage of disturbed renal function in adrenal insufficiency. Administration of Na salts to the adrenalectomized dog improves renal function by replacing the deficits of Na and Cl in the body and by increasing the urine volume. Complete restoration of renal function requires adrenal extract.—From Authors' Summary.

HERLANT, M. Gonadotropic power of the hypophysis in Addison's disease (Pouvoir gonadotrope de l'hypophyse dans la maladie d'Addison). *Compt. rend. Soc. de biol.* 129: 55. 1938.

One half of each of 2 pituitaries of 2 women who died of Addison's disease was dried in acetone, powdered, prepared in solution, and tested for gonadotropic potency in 2 immature female rats. The remaining half lobes were studied histologically. Each gland was found to possess gonad-stimulating power which was only slightly below that displayed by pituitaries of women dying from other diseases, yet histologically both were markedly deficient in basophiles. It was concluded that gonadotropic hormone may be produced by cells other than basophiles.—E. Cutuly.

HIPPLE, M. A. Results obtained with suprarenal concentrate in 10 cases of allergy. *Internat. Clin.* 2: 263. 1939.

The allergic manifestations in the patients described included bronchial asthma, herpes zoster, urticaria and dermatitis venenata. Suprarenal concentrate treatment by oral administration yielded consistently good results.—I. B.

INGLE, D. J. The effects of administering large amounts of cortin on the adrenal cortices of normal and hypophysectomized rats. *Am. J. Physiol.* 124: 369. 1938.

The purpose of the experiments was to determine whether or not massive doses of cortin (no assay given) would inhibit the adrenotropic activity of the anterior pituitary. Sixty male rats, weighing approximately 80 gm. each, were used. When normal rats were treated with large amounts of cortin, extensive atrophy of the adrenal cortices consistently occurred. When the pituitary body was absent and the animal received adrenotropic hormone in constant amounts there was no apparent effect of cortin on the adrenal cortex.—K. A. Brownell.

LEBLOND, C. P., AND G. SEGAL. Effect of colchicine on adrenals and lymphatic organs (Action de la colchicine sur la surrénale et les organes lymphatiques). *Compt. rend. Soc. de biol.* 128: 995. 1938.

Colchicine injected into rats caused adrenal hypertrophy and involution of the thymus. Histo-

logically the adrenal cortex showed loss of lipoids and the thymus revealed pycnotic atrophy. The thymus did not regress in adrenalectomized rats injected with colchicine.—E. Cutuly.

NUZUM, F. R., AND J. W. DALTON. Hypertension associated with lesions of adrenal glands. *Am. Heart J.* 16: 643. 1938.

This study summarizes the clinical instances of lesions of the adrenal glands which have been found to cause persistent or paroxysmal hypertension. To the list of 29 examples of change in the adrenal cortex which caused hypertension, another is added as an instance of hyperplasia of the right adrenal cortex which caused, in a premortem period of 5 months, a terminal rise in blood pressure to 180/100, in spite of the presence of definite cardiac decompensation. To the list of 85 instances of pheochromocytomas, another is added. This tumor caused typical paroxysmal hypertension in a man of 56 years. The diagnosis was made from clinical observation and roentgen studies of perirenal air injections. It was confirmed and the patient was cured by removal of the right adrenal gland. Charts of the clinical course of the patients, pictures of the roentgenograms and of the pathological material, and a complete discussion of the cases reported in the literature are included in this paper.—From *J. Nerv. & Ment. Dis.*

SEGAL, G., AND C. P. LEBLOND. Alarm reaction produced by x-ray on the abdomen of the rat (Réaction d' alarme produite par l'action des rayons X sur l'abdomen chez le rat). *Compt. rend. Soc. de biol.* 129: 279. 1938.

Irradiation of the abdomen induced a non-specific reaction characterized by atrophy of the lymphatic organs and hypertrophy of the adrenals. Atrophy of the lymphatic organs, however, did not occur if the rats were adrenalectomized.—E. Cutuly.

SHAY, H., J. GERSHON-COHEN AND S. S. FELS. The effect of duodenal stimulation in man upon alimentary and adrenalin hyperglycemia. *Ann. Int. Med.* 11: 1563. 1938.

Duodenal stimulation failed to prevent or decrease the hyperglycemia from adrenalin injection.—F. A. H.

THORN, G. W., R. P. HOWARD AND K. EMERSON, JR. Treatment of Addison's disease with desoxy-corticosterone acetate, a synthetic adrenal cortical hormone. *J. Clin. Invest.* 18: 449. 1939.

The use of oil injections of desoxy-corticosterone acetate daily into 8 patients with Addison's disease, without added salt, resulted in marked improvement, indicated by increased weight, elevation of blood pressure, restoration of plasma volume, Na, K, and Cl toward normal levels, retention of Na, increased excretion of K and P, and improved muscular strength. Substitution of a placebo injection resulted in prompt relapses. The daily doses needed varied from 2 to 30 mg.—E. L. S.

TOTH, L. A. Renal and vascular responses to epinephrine injections in glomerular and aglomerular fish. *Am. J. Physiol.* 126: 347. 1939.

Intravenous epinephrine injections varying in doses from 242 to 3γ per 100 gm. body weight in the puffer (*Spheriodes maculatus*) possessing glomerular kidneys resulted in a polyuria; similar injections in the toadfish (*Opsanus tau*) possessing aglomerular kidneys, in which the dose varied from 67 to 5γ per 100 gm. body weight resulted in no significant polyuria. Both species showed a rise in arterial blood pressure, but the heart rate of the puffer decreased significantly while the heart rate of the toadfish increased slightly. The hypothesis is advanced that the glomerulus with its afferent and efferent arterioles is necessary for the epinephrine polyuria to occur.—Author's Summary.

WESTERLUND, E. Clinical-experimental studies on hormonal regulation of fat resorption: Case of Addison's disease with change of fat tolerance curve following administration of adrenal cortex extract. *Nord. med., Helsingfors* 2: 1065. 1939.

The author believes that insufficiency of the adrenal cortex is a factor in the pathogenesis of the steatorrhea which may accompany Addison's disease. He describes a case in which there were pathologic amounts of fat in the feces. Administration of fat before the start of treatment with adrenal cortex extract showed a low fat tolerance curve. On repeated administration of fat after one injection of the extract and after protracted administration of the extract the fat tolerance curves were considerably higher.—From *J. A. M. A.*

WINTER, C. A., W. R. INGRAM AND E. G. GROSS. Effect of pitressin injections upon the serum electrolytes and water exchange of cats with diabetes insipidus and adrenal insufficiency. *Am. J. Physiol.* 126: 64. 1939.

Pitressin, injected subcutaneously in doses of 3 pressor units 3 times daily, was found adequate to control the polyuria of uncomplicated diabetes insipidus. Cats were kept on a constant diet. Animals with diabetes insipidus had normal serum Na, K, and Cl. Pitressin in the amount used did not affect serum Na, K or Cl of normal or diabetes insipidus cats. After adrenalectomy, untreated polyuric cats had a

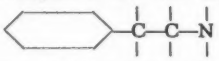
very short survival i.e., 3 to 5 days, and did not show the usual decrease in serum Na and Cl but if pitressin was administered, diabetes insipidus cats survived about as long as ordinary adrenalectomized cats and showed the usual changes in serum Na and Cl. The fall in these electrolytes in pitressin treated adrenalectomized cats with diabetes insipidus was not entirely due to the prolonged survival time. Ordinarily adrenalectomized cats lost more Na than Cl; adrenalectomized cats treated with pitressin lost Na and Cl in approximately equivalent amounts. Pitressin administration to adrenalectomized cats did not affect the survival. All adrenalectomized cats showed negative H_2O balance which was of about the same order of magnitude for non-polyuric cats with and without pitressin and for diabetes insipidus cats on pitressin but was much greater in the untreated polyuric cats. Total water loss in the adrenalectomized animals was slightly less in the latter group. In no case was the total H_2O loss great enough in itself to account for death.—K. A. Brownell.

Wyman, L. C. and C. T. Suden. Modification of adrenalin intoxication by adrenalectomy. *Am. J. Physiol.* 126: 7. 1939.

Single massive intraperitoneal doses (0.2 to 0.5 mg. per 100 gm. body weight) of adrenalin killed a majority of normal rats within an hour. Blood pressure and blood sugar remained elevated until death. Survivors recovered rapidly and permanently. Following adrenalectomy similar doses of adrenalin killed about the same proportion in not less than 3 to 24 hours. There was a slow fall in blood pressure and in blood sugar and an absence of pulmonary involvement. Animals that recovered survived a shorter time than that expected in adrenalectomized rats. The presence of cortical tissue was associated with acute symptoms which were more severe than normal. Fasting increased the susceptibility of both normal and adrenalectomized rats. Pretreatment with histamine made normal animals react more like adrenalectomized animals.—F. A. H.

Youmans, W. B., K. W. Aumann and H. F. Haney. Relation of the various groups of the adrenalin molecule to its intestine inhibiting function in unanesthetized dogs. *Am. J. Physiol.* 126: 237. 1939.

Compounds closely related to epinephrin possess similar pharmacological action as determined by their action on the intestine and on the nictitating membrane. Removal of any one of the groups of the epinephrin molecule that distinguish it from the fundamental sympathomimetic nucleus



results in a compound of less intestine inhibiting potency. The most important group is the meta —OH, next are the para —OH and the secondary alcohol —OH. The $-CH_3$ on the nitrogen is least important.—F. A. H.

ENDOCRINE GENERAL

Bromer, R. S. The value of roentgen-ray diagnosis in endocrine disorders in childhood. *Pennsylvania M. J.* 42: 1186. 1939.

The roentgenologist can offer assistance to the endocrinologist in the following conditions: congenital and juvenile hypothyroidism, thymic hyperplasia, infantile and juvenile thyropituitarism, hyperparathyroidism, gonadal disturbances such as hypergonadism, tumors of the adrenal body and other allied conditions.—I. B.

Cannon, W. B., and K. Lissak. Evidence for adrenaline in adrenergic neurones. *Am. J. Physiol.* 125: 765. 1939.

Tissue extracts from frogs, cats and other mammals were tested for the presence of adrenaline by the, blood pressure response, by the responses of the iris, the nictitating membrane and the uterus of the cat by the frog heart and by the specific chemical test of Shaw. Positive results with all these tests were obtained with extracts from adrenergic fibers, like mesenteric nerves, or organs containing this type of fibers, like the heart. Extracts of the vagus, skeletal muscle and the heart after degeneration of sympathetic fibers contained no adrenaline. Extracts of liver blood vessels contained an adrenaline-like substance, but liver pulp after removal of most of the vascular tissue was almost free of this substance. This observation is considered as significant for the question whether the sympathetic nerve fibers innervate the liver cells.—Emil Bozler.

Cohen, L. H. Psychiatric changes associated with induced hyperthyroidism in schizophrenia. *Psychosomatic Medicine* 1: 414. 1939.

The psychiatric changes produced in a group of 8 deteriorated schizophrenic patients given large dosages of desiccated thyroid (Armour's U.S.P.), up to 36 grains daily, over long periods of time have been studied. Although physiological changes, reported elsewhere, were considerable, general clinical evidence of hyperthyroidism was lacking. The psychiatric changes observed were confined almost

exclusively to the simple psychomotor activities. The findings are interpreted to indicate that, under such conditions, no qualitative change in mental activity is produced. But there is a quantitative increase in the repetition and perseveration of the primitive, highly-automatized activities which characterize the lives of deteriorated schizophrenics.—Author's Summary.

DUNN, C. W. Endocrine therapy in children. *Pennsylvania M. J.* 42: 1181. 1939.

Endocrine therapy in children is based on the following requirements: (1) a knowledge of the normal physiology of childhood and of the rôle played by the ductless glands in this physiology; (2) a complete constitutional and endocrine diagnosis; (3) a clear understanding of the preparations to be administereed. The nature and amount of endocrine therapy should be the minimal requirement of the child under treatment, as large doses may result in disuse atrophy of the glands involved. While primary hypothyroidism responds favorably to thyroid therapy, the secondary form resulting from primary anterior pituitary deficiency requires supplementary administration of the thyrotropic hormone of the anterior pituitary. The occasional child with myxedema refractory to thyroid extract may respond well to the oral administration of thyroxin. Conservative treatment of hyperthyroidism of childhood is useful. Cases of arrested or retarded growth may be of endocrine or nonendocrine nature. The use of growth hormones is as yet inconclusive. Oral administration of anterior pituitary occasionally succeeds when growth hormones fail. The injudicious use of A.P.L. in cryptorchidism is capable of producing macrophallus and edema of the scrotum. The use of adrenal cortical hormone in marasmus, as well as in the precollapse stages of severe toxic states such as diphtheria has reduced the mortality rate. Mumps may produce a severe toxic effect on the gonads, which manifests itself clinically a year or longer after the infection; the author therefore suggests that prophylactic and therapeutic serum treatment be employed in mumps whenever possible.—I. B.

EINHORN, N. H., AND L. G. ROWNTREE. Some endocrine factors controlling growth and development in childhood. *Pennsylvania M. J.* 42: 1183. 1939.

A number of factors such as nutrition, infection, mineral salts, vitamins and heredity are often concerned, and their rôle cannot be disregarded, in any discussion of growth and development. Both vitamins and hormones probably act as specific catalytic agents in the local and general growth processes which influence living cells to develop according to some less apparent controlling mechanism. At least 3 principles elaborated by the endocrine glands are concerned in growth and development. Two of these, a growth hormone and a sex hormone, are secreted by the anterior lobe of the pituitary gland. The third hormone elaborated by the thyroid is concerned not only with normal growth but also with development. Clinically, the growth hormone elaborated by the eosinophilic cells of the anterior pituitary has been used in the treatment of pituitary dwarfism with encouraging results. The gonadotropic hormones elaborated by the basophilic cells of the anterior pituitary, although having no direct influence on general growth, occupy a position of importance because of their influence on the growth of the sex organs. The thyroid hormone is concerned with growth but in a manner quite different from that of the pituitary growth hormone. The latter seems to stimulate general growth while the former is concerned with the differentiation of tissue. In the treatment of hypothyroid dwarfism, it is important that the dose of thyroid extract be pushed to the point of tolerance, that is, until rapid pulse, nervousness, or sweating are observed and the final dosage kept just below this point. These clinical signs are far more important in gauging the dosage of thyroid than is either the determination of the B.M.R. or roentgen-ray appearance of the epiphyseal centers. In the treatment of pituitary dwarfism the use of 3 substances is indicated—pituitary growth hormone, gonadotropic hormone and thyroid extract. The growth hormone and the gonadotropic hormone are not used simultaneously since it has been repeatedly pointed out that they are antagonistic to each other. In young children it seems advisable to treat the patient with growth hormone until maximum growth is attained or until it seems advisable to change to sex stimulation. In older children, it seems expedient to sacrifice stature to obtain adequate sexual development. Time is an important factor in the care of any patient suffering from a disorder of growth and development. As a general rule, several years of intensive treatment are necessary in order to accomplish noteworthy results. Three illustrative case histories are included.—I. B.

ELTERICH, T. O. Endocrinology in childhood. The pituitary in pediatric practice. *Pennsylvania M. J.* 42: 1172. 1939.

This is a very brief clinical discussion of the pituitary as it applies to the pediatrician's daily work, with the citation of 4 illustrative case histories. Whether the syndrome commonly known as Fröhlich's syndrome is due to a pituitary deficiency or to disturbances in the hypothalamic region of the brain is a debatable question. Elterich classifies 'big children' into 3 classes: those with simple obesity, those with large skeletons (adipositas gigantism—Opitz), and those presenting a combination of advanced somatic development with precocious sexual development due to excessive development of both acido-

philic and basophilic cells of the anterior pituitary. Large children may be further classified with regard to intelligence. Some are normal, others even of superior intellectual capacity, while in some the pituitary manifestations are a part of a more general underdevelopment of the brain. Big children must therefore be appraised from the viewpoint of height, weight, skeletal ripening, sexual maturity, intellectual capacity and associated brain pathology. Finally the relationship of the pituitary to diabetes insipidus and diabetes mellitus must be borne in mind.—I. B.

Evans, F. A. Nature of obesity in endocrine disorders. *Pennsylvania M. J.* 42: 1174. 1939.

Obesity can result only from taking in more energy components than are expended. Obesity associated with endocrine disorders yields to dietary measures in the same manner as that without accompanying evidence of endocrine imbalance. Indeed it can almost be said that it will not yield to anything but dietary control. Obesity in endocrine disorders is not always secondary; it and the endocrine symptoms may both be due to the ingestion of a faulty metabolic mixture in childhood or at puberty, physiologically impressionable ages. Endocrine imbalance when primary can cause obesity only by decreased energy expenditure, or increased energy intake because of abnormalities of appetite. Abnormalities of appetite might well result in many cases also from faulty habits of eating, or a hereditary tendency as suggested by Wilder. Whatever the background of the abnormal appetite leading to overeating, which can be the only cause of obesity, although increasing the difficulty of dieting, it does not, even when a hereditary tendency, justify the continuation of an unwholesome practice.—I. B.

Fliess, M. N., and S. M. Bloom. Von Gierke's glycogen storage disease; case reports with unusual findings. *J. Ped.* 12: 635. 1938.

This is the ninth case of von Gierke's disease to be reported in the American literature. The other 8 were equally divided into the cardiomegalic and hepatomegalic types. The present case which presented unusual symptoms was in a 26-month-old girl who gave a history of pallor and difficult feeding. She developed teething and talking at about the normal time but had never walked. On examination, she showed a somewhat mongoloid facies with extreme pallor, hemoglobin of 15%, red blood count of 810,000 cells, enlargement of the heart, liver and abdomen. Nephrotic edema developed in a few days accompanied by albuminuria, hypercholesteremia (500 mg./100 cc.). The liver receded when the edema was present. A rapid apparent cure occurred under the treatment of liver extract, transfusion of blood and iron and pitressin.—M. B. G.

Gurin, S., C. Bachman and D. W. Wilson. The gonadotropic hormone of urine of pregnancy. I. A simple method of extraction and purification. *J. Biol. Chem.* 128: 525. 1939.

Pregnancy urine was treated with benzoic acid and the precipitate washed several times with saturated aqueous benzoic acid solutions. The resulting material was treated with acetone to remove the benzoic acid and when dried was found to contain 25 to 50 minimal effective doses (m.e.d.) per mg. by assay on mature rabbits. A single extraction of this product with 30% aqueous acetone and subsequent re-precipitation from 70% acetone increased the potency to 500 to 2000 m.e.d. per mg. Extraction of the first crude product with 50% ethanol at pH 6.0 and re-extraction with same solvent at pH 4.8 gave a preparation having 1000 to 3000 m.e.d. per mg. A still higher purity could be obtained by dialysis or treatment with tannic acid, or iodine in potassium iodide. The hormone was soluble in glycerol, ethylene glycol, ethylene chlorohydrin and formamide. It is stable in the dry state but loses much of its activity when kept in aqueous solution even in the cold.—J. M. L.

Gydesen, C. S., and B. J. Murphey. Some endocrine dyscrasias associated with conduct disorders in Colorado Springs children. *Rocky Mountain M. J.* 36: 462. 1939.

In view of the contradictory and confusing reports regarding the relationship, or lack of relationship, between behavior disorders in children and the various types of endocrine dyscrasias, the authors reviewed 64 cases that had been examined by a child psychiatrist in a child guidance clinic and also by an endocrinologist in private practice. Thirty-six of these hcildren were referred to the endocrinologist by the child guidance clinic as cases presenting a possible endocrine dyscrasia. The 36 cases were selected from a series of 313 consecutive full-study cases examined in the child guidance clinic. Thyroid (32) and pituitary (18) disorders were by far the most common conditions discovered. All the children specifically treated with endocrine therapy showed definite improvement in their school and social adjustment.—From abstract, *J. A. M. A.*

Klein, J. R. Effect of thyroid feeding and thyroidectomy on the oxidation of amino acids by rat kidney and liver. *J. Biol. Chem.* 128: 659. 1939.

The rate of oxidation of d-amino acids by minced cell preparations of liver and kidney from control, thyroid fed and thyroidectomized rats was measured in the Warburg apparatus. The increase in rate of metabolism produced by feeding thyroid was accompanied by an increase in d-amino acid oxidase in the

liver but not in the kidney. Thyroidectomy caused a decrease in the oxidase in the liver but had no effect on the kidney.—J. M. L.

KOKUBO, Y. How will sexual cycles of healthy women influence blood picture? *Tohoku J. Exper. Med.* 35: 243. 1939.

Kokubo studied the blood picture of 3 unmarried healthy women once every week during a period of 3 months. He found that the features common to all were as follows: the red cell count decreased in the premenstrual period and fell to a minimum in the menstrual period; the hemoglobin decreased from the premenstrual through the menstrual toward the postmenstrual period; Arneth's nuclear shift of neutrophiles was almost unaffected by sexual cycles; pathological granules were inclined to decrease in the menstrual period; the hemosedimentation was normal generally throughout the sexual cycles but tended to decrease in 1 to 2 weeks after menstruation began. The platelet count fluctuated regularly in the way apparently characteristic of each individual.—Margaret Baptist.

KOSTER, L., AND R. DE BOER. Further observations on hypophysio-endocrine reactions in tuberculous infections. *Nederl. tijdschr. v. geneesk.* 82: 4062. 1938.

Koster and de Boer in 1935 described a syndrome which occurs in tuberculosis and which is characterized by obesity, erythemia, hypertension, purplish red striae, hyperglycemia, glycosuria, hyperthermia, moderate increase in the basal metabolism and genital anomalies. Because these symptoms are the same as those that have been observed in hypophysial disorders, hypophysial anomalies have been regarded as a causal factor. In the course of the last several years the authors observed additional cases, and in this report they review fourteen in all. They investigated especially the cholesterol ester rate and the lipase content of the serum of these patients. They found that these values are increased in these patients, which is an indication of a hyperfunction of the hypophysis or at least of some of its elements. That this hypophysial disorder is especially frequent in tuberculosis the authors regard as a result of the fact that the immunity reactions of tuberculosis are entirely different from those of other infections. They consider the hypophysial hyperfunction as a form of defense reaction.

SURE, B., R. M. THEIS AND R. T. HARRELSON. Vitamin inter-relationships. I. Influence of avitaminosis on ascorbic acid content of various tissues and endocrines. *J. Biol. Chem.* 129: 245. 1939.

White rats were fasted or fed on diets deficient in Vitamins B_1, B_6, *A*, or riboflavin. No changes in the ascorbic acid content of the endocrine glands were found in the fasted animals in vitamin B_6 deficiency nor after feeding 4 to 16 mg. ascorbic acid daily. Vitamin B_1 deficiency caused a 27.8% reduction in the weight of the thymus. Vitamin A deficiency caused the following weight losses, thymus 19.2%, adrenals 12.4%, pituitaries 25.8% and thyroid 15.3%. Riboflavin deficiency caused the most marked losses as follows, thymus 85.7 and thyroid 20.7 but did not affect the adrenals or pituitaries.—J. M. L.

WORTIS, J. The management of shock treatment of psychoses. *M. Clin. North America* 23: 797. 1939.

Shock treatment permits of the following variations or modifications of procedure: (1) Variations of shock type in which, if deemed desirable, insulin shock seizures may be modified by such anticonvulsants as the barbiturates or the seizures may be induced or increased by the supplementary use of metrazol; (2) variations in insulin dose with a daily increase by 5 to 20 u until coma is induced in the 3rd or 4th hour after the injection with subsequent use of a minimum shock dose; (3) variations in duration of hypoglycemia in which, in feeble patients, hypoglycemia may be terminated in an hour or two, or it may be terminated at any feasible time (deep coma should not extend beyond an hour and a half); (4) variations in method of termination of hypoglycemia—by feeding with a cup, or with stomach tube, or by intravenous glucose administration, by epinephrine injections, or by a combination of these; (5) the use of auxiliary drugs such as thyroid extract or by vitamin B_1. Since insulin treatment admits of more variation and individualization and is less likely to produce brain damage it is probably preferable to metrazol, the exception being in cases of catatonic stupor. This paper is of such length and importance that it does not lend itself to further abstracting.—I. B.

GONADS

ASTWOOD, E. B. Changes in the weight and water content of the uterus of the normal adult rat. *Am. J. Physiol.* 126: 162. 1939.

Determinations on the weight and water content of the uteri of adult rats during closely graded stages of the estrous cycle and during pseudopregnancy show that the uteri reach a maximum size at proestrus and then regress to a minimum on the first day of diestrus. Their water content rises to a maximum before proestrus and then falls abruptly with the first appearance of cornified cells in the vaginal smear. Uteri of pseudopregnant animals follow the same course for the first 3 days, show an increase in weight and water content of the 5th day, and then regress below the diestrous level. The uteri of animals in various stages of the cycle, given a single injection of 2.0r estradiol 6 hours before death, responded by

a marked increase in tissue water during diestrus and proestrus. During proestrus and estrus this reaction was strongly inhibited. Evidence is presented showing that this inhibition is due to an ovarian secretion having the properties of a corpus luteum hormone. The events are taken to mean that estrogen acts unopposed during a brief period before proestrus and largely ceases to be produced prior to vaginal cornification. Corpus luteum hormone acts upon the uterus during proestrus and estrus; it is released from ovarian follicles during their preovulatory swelling and ceases to be produced at or shortly after ovulation—Author's Summary.

Benedek, T., and B. B. Rubenstein. The correlations between ovarian activity and psychodynamic process: I. The ovulation phase. *Psychosomatic Medicine* 1: 245. 1939.

Independent studies were made of the instinctual trends and of the vaginal smears and rectal temperatures of 9 women through a total of 75 menstrual cycles. The psychological studies depended upon psychoanalytic procedures and especially dream analysis. After 10 months the psychological and the physiological interpretations were compared, disclosing a high degree of correspondence. It was thus shown that the follicular phase of the cycle is characterized by active heterosexual libido. This appears normally as a wish for heterosexual gratification but may turn into aggression or a fearful, defensive attitude. The psychodynamic orientation is toward the male. The period of corpus luteum dominance is characterized by erotization of the female body, the woman becoming more passive and dependent. Thus ovulation is marked by sudden decrease of estrogenous activity and the influx of narcissistic erotization. Emotionally this is manifested as relaxation of the preovulatory tension which had been caused by the conflicting tendencies of the increased estrone and incipient lutein activity. In general, it appeared that whenever the metabolic gradients that are correlated with the specific activities of the two hormones change in direction or slope the psychoanalytic material shows a corresponding change in instinctual drives. Extension of the method is suggested as desirable for further researches on the biological foundations of instincts.—R. G. H.

Benoit, J. Rôle of the eyes and ocular-hypophyseal nerve pathway in gonadal stimulation by artificial light in the domestic duck (Rôle des yeux et de la voie nerveuse oculo-hypophysaire dans la gonadostimulation par la lumière artificielle chez le canard domestique). *Compt. rend. Soc. de biol.* 129: 231. 1938.

Stimulation of the testes of immature drakes has been produced by illumination of the orbital region. The results of this study support the view that the illumination stimulates the pituitary through autonomic fibers traveling to the pituitary from the retina by way of the optic nerve and hypothalamus.—E. Cutuly.

Benoit, J. Eunuchoidism by hypoplasia or absence of interstitial cells in cocks (Quelques cas d'eunuchoïdisme par hypoplasie on absence de tissu interstitiel glandulaire chez le coq domestique). *Comp. rend. Soc. de biol.* 129: 234. 1938.

Five castrated cocks with combs showing little or no stimulation were found to have regenerated large testicular masses. Histological examination of these masses revealed few or no interestitial cells, but large and fully developed tubules. These data were regarded as further proof of the production of male hormone by the interstitial cells and as findings unfavorable to the view that interstitial cells are trophic to the germinal cells.—E. Cutuly.

Boycott, M., and I. W. Rowlands. The biological nature and quantitative variation of the gonadotropic activity of pregnant women's serum. *Brit. M. J.* 1: 1097. 1938.

A large number of samples of serum were obtained from pregnant women and assayed for gonadotropic activity, a bulk preparation from pooled samples being used as a standard of comparison and for determining the general gonadotropic properties. In contrast to pregnant mare serum extract, extracts of human pregnancy serum behave similarly to extracts of urine of pregnancy, in that they cause only a limited reponse in the rat ovary (judged by increase in ovarian weight) and they produce no follicular growth in the ovary of the hypophysectomized rat. Assays on the individual samples of serum taken at different stages of pregnancy show that the concentration of gonadotropic substance rises rapidly from the 6th week, is at a maximum from the 8th to the 12th week (250% gonadotropic activity), and thereafter declines to a low level, which is fairly constant, from the 20th week to the end of pregnancy (50%). By the technic of assay employed no gonadotropic activity could be detected in the serum of nonpregnant women.—H. J. D., Jr.

Bruck, S., and J. M. Fruchter. Roentgen treatment of menstrual dysfunctions and sterility: analysis of 108 cases. *Radiology* 32: 446. 1939.

The authors discuss the endocrine factors governing the normal menstrual cycle and also the endocrine imbalance in the production of menstrual dysfunctions and sterility as it is related to the pituitary and ovary. They treated 108 cases of various menstrual dysfunctions (amenorrhea, oligomenorrhea,

menorrhagia and metrorrhagia) in single and married women, with and without sterility, with roentgen rays. From their summary of the results obtained they believe that they are justified in stating that, although this treatment is empirical and its mode of action is not yet understood, it is definitely worth while and gives a fifty-fifty chance to those women in whom all other methods have been tried and found wanting. Pregnancy took place in 45% of women with deficiency menstruation and sterility.—From J. A. M. A.

Bulliard, H., and I. Grundland. Estrogenic ability of proteids (polypeptides) [Sur le pouvoir oestrogenè des protides (polypeptides)]. *Compt. rend. Soc. de biol.* 128: 997. 1938.

Injection of a solution of polypeptides into 12 female mice 3 to 4 weeks old is said to have induced premature vaginal canalization, stratification of the vagina and uterus, and mucus secretion in the vagina. Vaginal cornification did not occur. The estrogenic effects were said to indicate the need for proper identification of substances giving estrogenic reactions.—E. Cutuly.

Burns, R. K., Jr. The effects of crystalline sex hormones on sex differentiation in amblystoma. I. Estrone. *Anat. Rec.* 71: 447. 1939.

Repeated injections of estrone into larval Amblystoma punctatum beginning shortly after the onset of sex differentiation in the gonads, causes transformation of the testes of genetic males into ovaries. Comparatively few testes retain more than traces of their original character. The development of genetic ovaries, however, is not appreciably accelerated. Controls studied histologically at the beginning and at the end of the experiment showed the usual 1:1 sex ratio, without unusual manifestations of larval bisexuality. The developing oviducts of normal larvae during the period of the experiment are incomplete and rudimentary in their differentiation, with no sexual dimorphism. Such oviducts are capable of responding precociously to estrone in the dosage given. There was increase in both length and diameter of the ducts irrespective of sex, which is independent of reversal stages in the gonad and may be attributed to direct action of the hormone. There was no observable effect on the wolffian ducts and it is concluded that these prospective male ducts are indifferent to the presence of estrone. There was no growth of the cloacae. In this experiment estrone behaves in larval Amlbystoma as in the developing chick, specifically promoting cortical growth in the gonad and accelerating development of the female ducts in both sexes: but has no demonstrable influence on male parts.

Cacciarelli, A. The inhibition of lactation during puerperium by anterior pituitary and ovarian extracts. *Am. J. Obst. & Gynec.* 38: 149. 1939.

The author used unstandardized extracts of ovary, or of ovary and pituitary (said to contain no estrin). Results were said to be better with the mixture. Condensed tabulated results are presented on 25 cases from 75 in which series excellent results are claimed in 90%.—E. L. S.

Caffler, P., and E. S. Oeskayaalp. The excretion of stilbene preparations (Zur Frage der Ausscheidung der Stilben-Praparate). *Zentrlbl. f. Gynak.* 63: 1218. 1939.

Using rabbits the authors found rapid excretion of stilbene material when injected, employing castrate mice as recipients for untreated urine. The responses were graded into 10 stages, from castrate to full estrous response. The smallest recognizable reaction is produced by use of 0.005 γ. The preparations are "cyren" or its acetate or propionate. The esters are assumed to have a prolonged activity. Excretion can be detected within 5 hours in the human, and after a single dose of 2.5 or 5 mg. it continues for 10 to 16 days. The authors think diuresis accelerates excretion (only two cases tried). Maximum excretion seems past by the 5th day, suggesting this as the time for repeated injection if sustained effects are desired. The total amount recoverable from urine is not reported, but it appears that from the 5th to 11th days after 5 mg. about 7.5% of the dose appeared.—E. L. S.

Campbell, R. E., and E. L. Severinghaus. Pituitary Gonadotropic extracts for treatment of amenorrhea, menorrhagia, and sterility. *Am. J. Obst. & Gynec.* 37: 913. 1939.

Samples of typical results are presented from a 7 years experience in treating various syndromes with standardized pituitary preparations. Amenorrhea, menorrhagia, irregular menses and sterility are treated as the consequences of ovarian underactivity. Vaginal epithelium, pregnandiol excretion and especially endometrial biopsies are the criteria used in diagnosis and therapy. The use of repeated series of daily doses, for from 5 to 15 days in the first weeks of the menstrual cycles, are considered necessary for optimum results.—E. L. S.

Caridroit, F., and V. Régnier. Determination of maintenance threshold of the comb of the domestic cock (Détermination du seuil de conditionnement total de la crête du coq domestique). *Compt. rend. Soc. de biol.* 129: 445. 1938.

The amounts of testosterone propionate necessary to maintain the combs of 4 cocks at a size level existing just before castration ranged from 0.3 mg. to 1.7 mg. daily. In another capon the comb main-

tenance dose was 0.1 mg. daily; but when the comb was allowed to regress, only 0.02 mg. was necessary to initiate comb growth.—E. Cutuly.

CASTILLO, E. B., AND A. PINTO. Effect of estrone and testosterone on the male genital organs (Action de l'oestrone et de la testostérone sur l'appareil genital masculin). *Compt. rend. Soc. de biol.* 129: 868. 1939.

Prolonged injection of estrone, 15 U daily for 1 to 4 months, produced varying degrees of atrophy of the prostate and seminal veiscles in 30 rats. Hypertrophy of these organs in rats and guinea pigs followed short treatments, 50 U of estrone for 12 days. In castrated rats and guinea pigs, involution of the prostate and seminal vesicles were retarded by estrone. Testosterone caused an enlargement of these organs in castrates, while a combination of the two hormones produced a marked hypertrophy.—J. C. D.

COURRIER, R., AND G. GROS. Action of folliculin in the male cat (Sur l'action de la folliculine chez le chat male). *Compt. rend. Soc. de biol.* 129: 8. 1938.

Male monkeys about one year old and newborn or 20-day-old male cats were grouped so that some were castrated and some were intact. All animals were injected daily for 20 days with 0.2 mg. of dihydrofolliculin. In the monkeys the hormone caused development of only the interglandular substance of the accessory sexual organs. In the cats, castrated or intact, the hormone stimulated the glandular elements proper of the accessory organs and, in addition, induced epidermoid metaplasia of the prostatic utricle and urethra. The male hormone, testosterone propionate, it was mentioned, caused uterine hypertrophy as well as hypertrophy of the clitoris in the immature hedgehog.—E. Cutuly.

D'AMOUR, F. E., D. FUNK, AND H. LIVERMAN. Daily Gonadotropic hormone tests during fifty complete menstrual cycles. *Am. J. Obst. & Gynec.* 37: 940. 1939.

Daily tests were made on 24-hour urine samples from 12 women, in 50 complete cycles, using a tannic acid precipitation method and the injection into 26-day-old rats. The occurrence of positive responses is assumed to indicate ovulation, and the appearance of anovulatory cycles and also of multiple ovulations in single cycles is discussed.—E. L. S.

DANTCHAKOFF, V. Effects on procreation and progeny in guinea pigs subjected to testosterone treatment during development (Effet du traitement hormonal de l' embryon de cobaye par la testostérone sur ses facultés procréatrices et sur sa progéniture). *Compt. rend. Soc. de. biol.* 128: 891. 1938.

Successful procreation in mammals demands that the two sexes be properly constituted with respect to psychic behavior, accessory sexual organs and gametes. These factors are discussed on the basis of studies made on guinea pigs. Genetically female guinea pigs may be masculinized before or after birth by means of testosterone. Such animals will behave like genetic males as long as male hormone is injected into them; they can not themselves produce male gametes. At an early stage in development, male hormone will stimulate the formation of male accessory sexual organs and suppress development of normal accessories in genetic females. Male hormone injected into pregnant females as early as 18 days after copulation causes all young to be born with the external genitalia of males. However, genetic females possess ovaries and genetic males possess testes, even though the gonads in each instance may not be quite normal.—E. Cutuly.

DANTCHAKOFF, V. Use of the Allen-Doisy test in embryo lizards of both sexes (Sur la validité du test d' Allen et Doisy dans l'embryon de lézard des deux sexes). *Compt. rend. Soc. de biol.* 128: 895. 1938.

This study emphasizes the range of activity of follicular hormone in the animal kingdom. The hormone administered to embryo lizards caused keratinization of the terminal end of the cloaca and of a vesicular sac (of unknown function) situated in the abdomen. There occurred, also, sexual inversion of males.—E. Cutuly.

DANTCHAKOFF, V. Testosterone and the development of the sex organs in the embryo (Action de la testostérone et édification dans l'embryon de complexes d'organes sexuels). *Compt. rend. Soc. de biol.* 128: 1119. 1938.

Injections of male hormone were made directly into guinea pig embryos. The hormone had a decidedly strong masculinizing action in genetically female embryos before day 24 of development, but an inconstant effect between days 24 and 32. After day 32 male hormone induced only hypertrophy of the clitoris. The results are used as a basis for discussion of some phases of development.—E. Cutuly.

DANTCHAKOFF, V. Note on the "Unorthodox" gonadotropic properties of testosterone (Sur les proprietes gonadotropes paradoxales de la testostérone). *Compt. rend. Soc. de biol.* 129: 946. 1938.

The paradoxial gonadotropic ability of the male hormone to stimulate female reactions in gonads of female guinea pigs is due to the bisexual potentialities of the germ cells which, by their nature, are cells

open to two types of stimulation and capable of a double response; to the original location of the germ cells in tissues which are female as the result of genetic determination; and to the inability of a hormone of the opposite sex to disturb a tissue combination determined by the genetic inheritance.—Author's Summary.

Desmarest and Capitain. Value of testosterone therapy in metrorrhagias and menopausal disorders. *Presse méd.* 47: 1031. 1939.

The authors report the significant results achieved by means of testosterone in the therapy of metrorrhagias, singling out 5 of their numerous cases for clinical comment. These selected patients were divided into two groups: 3 young women between 17 and 30 years of age with menstrual and intermenstrual hemorrhages due to a uterine trauma and 2 women of 50 suffering from menopausal bleeding. The therapy, suggested by unexpected favorable secondary results in the treatment of mastopathies, consisted in medication with 10 mg. of testosterone at varying intervals of time and in decreasing amounts, as determined by the needs, and was not employed until after careful examinations for possible local or general pathogenic conditions. Testosterone therapy, successful as a hemorrhastatic agent, had a remarkable additional effect on the lipodystrophy and ovarian cyst of one of the patients in the first group but did not arrest disorders of the sympathetic nervous system in cases of menopause. In postmenopausal disorders the authors say that they successfully employed a combination of estrogen and testosterone discriminatingly administered in small graduated doses with time spacing, after an investigation in each case of existing dysfunction in ovarian and hypophysial activity. The authors recommend the simplicity and effectiveness of the combined therapies in stabilizing menopausal and postmenopausal stress and strain.—From abstract, J. A. M. A.

Dodds, E. C., and J. D. Robertson. Masked hypothyroidism as cause of amenorrhea. *J. Obst. & Gynaec. Brit. Emp.* 46: 213. 1939.

The authors report 5 cases of secondary amenorrhea resistant to simple substitution therapy. The patients presented none of the usual features of generalized disorders known to cause amenorrhea. Determination of the basal metabolism showed that quite a marked degree of hypothyroidism was present, despite the absence of any of the classic signs of this disorder. Adequate treatment with thyroid extract caused the basal metabolism to rise to normal and this was accompanied by the establishment of normal menstruation. In 1 case in which the dose of thyroid extract was reduced and the basal metabolism as a result fell below normal, amenorrhea reappeared, but menstruation again returned when an adequate dose of the thyroid extract was given to raise the basal metabolism to normal.—From abstract, J. A. M. A.

Dryerre, H. W. The diagnostic significance of the sodium and chlorine content of the blood and urine. *Edinburgh M. J.* 46: 267. 1939.

Various tests for Addison's disease based on the changed sodium or potassium metabolism are discussed. The test for adreno-cortical insufficiency proposed by Cutler, Power and Wilder has been carried out on 14 cases. Four of these were definitely suffering from Addison's disease, 5 were suffering from asthenia not due to Addison's disease, and the remaining 5 were normal healthy controls. The results reveal that on the 3rd day of the administration of a salt-poor high K diet the concentration of Na in the urine is much higher in cases of adrenal insufficiency than in control subjects. Under the conditions of the test the urinary chlorine concentration of the patients with adrenal insufficiency did not differ from that of the control cases with the consistency that was claimed by Cutler, Power and Wilder. Reference is made to a possible danger in carrying out the test in patients suffering from Addison's disease. The concentrations of Na and Cl in the blood serum of patients with Addison's disease may not differ materially from those of control cases.—From the Author's Summary.

Dynes, J. B. Estrogenic therapy of involutional melancholia. *Arch. Neurol. & Psychiat.* 42: 248. 1939.

Seven patients with involutional melancholia were treated with large doses of estradiol benzoate, given intramuscularly. The effectiveness was tested at weekly intervals by examining vaginal smears, determining the pH of the vaginal secretions and observing the reaction of the vaginal mucosa to compound solution of iodine. The patient's improvement or failure to improve was correlated with the changes indicated by these three tests. Except in 2 cases clinical improvement was definitely correlated with demonstrated effectiveness of the estrogen. Lessening and abolition of the acute agitation and tension were noted to occur in from 2 to 5 weeks. The failure of this type of therapy in 2 cases was attributed to irreversible physiologic changes. An outline is given of the schedule of treatment followed. No criteria, either physical or mental, foretell with certainty what benefit will result from estrogenic therapy. It is believed that every patient with involutional melancholia should be given a trial, an adequate dose of the estrogenic substance being used and its activity checked by observation of the changes in the vaginal smear, the pH of the vaginal secretions and the stain of the vaginal mucosa with compound solution of iodine.—Author's Summary.

Effkemann, G., and H. Strüver. The sympathicotropic activity of follicle hormone (Die sympathikotrope Wirkung des Follikelhormons). *Zentrlbl. f. Gynäk.* 63: 1248. 1939.

After extensive literature citations the authors propose to evaluate the effects of estrogens by photographic recording of the pupillary accommodation of the cat exposed to constant light stimulus, when under morphine, atropine, or d-pseudoephedrine, with and without pretreatment with estrogen. The graphic records show greater dilatation of pupils with estrogen pretreatment, the effect being most marked with morphine, slightly less with atropine. The pupil was larger from estrogen alone, before use of the mydriatic drugs.—E. L. S.

Engelhart, E. The induction of menstruation in castrate women by the action of a synthetic estrogen (Der Aufbau der Proliferationsschleimhaut bei der kastrierten Frau durch einen synthetischen oestrogenen Wirkstoff). *Wien. klin. Wchnschr.* 51: 1356. 1938.

Diethyl-stilbestrol, which had been shown to have active estrogenic action in animals, was shown to be active in causing the breakdown of the proliferation phase of the uterine mucosa of castrate women. Quantitatively it exceeded the activity of the naturally occurring follicular hormones—H. J. D., Jr.

Fels, E. Inactivation of estrone in the organism (Investigaciones experimentales sobre la inactivación de la hormona en el organismo). *Boll Acad. Nac. Med. Buenos Aires.* 867. 1938.

In castrated female rats 200 I.U. estrone injected subcutaneously or intramuscularly induced estrus in 15% of the cases (15 in 38), intracardiac gave 35% (12 in 34), into the vena cava or the portal vein 24% (8 in 34), into the spleen 24% (11 in 46) and into the liver 23% (11 in 48). Trauma was not the causa of reduction in the efficiency of the estrone, since estrus was not prevented by injecting saline into the different viscera simultaneously with the subcutaneous or intramuscular injection of estrone. Subcutaneous implantation of liver or spleen into which had been injected a dose of estrone which produced estrus in 100% of animals, did not reduce the potency of the hormone. Intrasplenic injection of 200 I.U. estrone followed by venous ligature up to 4 hours after, produced estrus in all cases. It is deduced that the liver and spleen inactivate estrone, but only when the tissue is functionally active.—J. T. L.

Fontès, J., B. Pereira and K. Gomes. Rhythmic movements of the rabbit uterus under the influence of estrone and pituitrin (Mouvements rhythmiques de l'utérus de la lapine sous l'action de l'eostrone et de la pituitrine). *Compt. rend. Soc. de biol.* 129: 693. 1938.

Data are presented to support the view that rhythmical contractions of excised rabbit uteri are caused by estrone under the influence of pituitrin.—E. Cutuly.

Gayet, R., L. Cuny and D. Quivy. Comparison of value of different assays in rats for pregnancy urine extracts (Valeur comparative des différents tests adoptés chez le rat pour doser l'activité des extraits d'urines de femme gravides). *Compt. rend. Soc. de biol.* 129: 197. 1938.

Of many methods of assay for pregnancy urine uterine growth in rats was found to be most acceptable. A rat unit was defined as the smallest amount producing a maximal response in the uterus, weighed dry, of immature rats injected 3 times at 24-hour intervals and autopsied 96 hours after the first injection.—E. Cutuly.

Gayet, R., L. Cuny and D. Quivy. Variations in weight of genital organs relative to body weight in immature rats and their importance in the biological assay of gonad-stimulating substances (Les variations du poids des organes génitaux en fonction du poids du corps chez les rats impuborès; leur importance dans l'essai biologique des substances gonado-stimulantes). *Compt. rend. Soc. de biol.* 129: 261. 1938.

It was stated that assays of gonad-stimulating substances were more accurate if natural variants, such as body weight, were considered in the analysis of results.—E. Cutuly.

Gesell, A., H. Thoms, F. B. Hartman and H. Thompson. Mental and physical growth in pubertas praecox. Report of fifteen years' study of a case. *Arch. Neurol. & Psychiat.* 41: 755. 1939.

A case of well defined uncomplicated pubertas praecox is described. The subject, a Jewish girl, showed physical signs of pubescence in the first year of life and menstruated at the age of 3 years and 7 months. She died at the age of 18 years and 7 months. Physical and mental measurements for 15 years are summarized and interpreted, with special reference to their developmental significance. Carpal roentgenograms showed marked precocity of skeletal growth. Growth in height practically ceased at 11. After brief initial irregularity, menses were regular until 13. The subsequent irregularity was suggestive of menopausal factors. The pelvis was of a typical dolichopellic type and posteriorly exceptionally spacious, suggesting an early osseous set combined with selective endocrine molding influences. Psychologic measurements made at 16 consecutive intervals showed a rather remarkable approximation to a near average course of intellectual development. The emotional life and personality manifestations were only slightly atypical. The stability of the mental development is interpreted as denoting a preferential im-

munity of the central nervous system to certain forms of endocrine imbalance. The developmental distortion of the precocious pubescence was most conspicuous in the skeletal system. Cranial development, however, followed an average course. Growth is regulated by specific determiners. The evidence afforded by pubertas praecox indicates that there is no single pervasively controlling sex factor.—Authors' Summary.

Giedosz, B. Increasing the effect of gonadotropic hormone with vitamin C (Sensibilisation de l'action de l'hormone gonadotrope par la vitamine C). *Compt. rend. Soc. de biol.* 129: 339. 1938.

Rabbits injected simultaneously with vitamin C and gonadotropic hormone responded with greater ovarian stimulation than rabbits treated with gonadotropic hormone alone.—E. Cutuly.

Guthrie, M. J., and K. R. Jeffers. The ovaries of the bat myotis lucifugus lucifugus after injection of hypophyseal extract. *Anat. Rec.* 72: 11. 1938.

Bats of the genus Myotis which normally ovulate late in March and in April when they leave their winter quarters in caves will sometimes ovulate spontaneously during February and March when brought from the caves to normal room temperatures. They can be induced to ovulate as early as December 12th by injection of hypophyseal extract. In January and February, 19 out of 20 females injected ovulated; the doses in all cases were relatively small. Normal development of ova occurs in bats stimulated by injections to ovulate during February, but no reliable instance of typical development has been obtained after ovulation induced in December and January. This is taken to mean that during the hibernation period sperm from autumnal inseminations probably lose the capacity to initiate normal development. There is a conspicuous increase in the relative number of smallest growing follicles after the injection of gonadotropic hormone. Growth of follicles beyond the size at which degeneration normally begins and the formation of antra are observed after treatment with extract. Growth and cytoplasmic differentiation of the oöcyte occur more slowly, in relation to growth of the follicle, in treated than in control bats. Injection of the gonadotropic hormone tends to inhibit the formation of meiotic spindles in mature oöcytes of retrogressing follicles.

Herlant, M. Gonadotropic power of the hypophysis of rats injected with insulin (Pouvoir gonadotrope de l'hypophyse de rats soumis a des injections d'insuline). *Compt. rend. Soc. de biol.* 129: 57. 1938.

Injections of insulin in rats caused multiplication and hypertrophy of the eosinophilic cells of the pituitary. The gonadotropic activity of the pituitaries of 15 insulin-injected rats was compared with that of normal glands by the method of implantation into immature female rats. Pituitaries of insulin-treated rats produced heavier and more intensely luteinized ovaries than the control glands. It was concluded that eosinophiles share a part in the production of gonad-stimulating hormone.—E. Cutuly.

Hoffman, W. J. Hormone therapy of male breast hypertrophy. *Am. J. Cancer.* 36: 247. 1939.

In view of the possibility that hypertrophy of the male breast might be due to a deficiency of the male sex hormone, Hoffman in 1935 treated 6 patients by injections of gonadotropic substance. Injections were given at weekly intervals over periods ranging from 2 to 10 months. No consistent results were obtained, and the experiment was abandoned after one year. In 1937 an experiment was begun to determine the effect of injections of male sex hormone into 31 boys or men with unilateral or bilateral hypertrophy of the breast. Testosterone acetate and propionate were the preparations employed. The youngest patient was 11 and the oldest 73 years of age. Twenty-eight of the 31 patients, with various degrees of hypertrophy, received injections twice a week. The remaining 3 patients, who were the youngest in the group, with the smallest masses of mammary tissue and a history of beginning regression, were excluded from the experiment. The dosage employed in the first 6 patients was 5 mg. of testosterone acetate twice a week. After 6 months the dosage in subsequent cases was increased to 25 mg. twice a week, when it became evident that the smaller dosage produced no testicular damage or arrest of the normal changes of puberty. Among the 28 patients who received injections of testosterone there was complete regression of the mass in 14, while the degree of regression exceeded 75% in 9. The elapsed time before complete regression occurred varied from 2 to 5 months, and the number of injections averaged 28. In general the response was relatively rapid in the beginning. It was not unusual to note a regression of 50% during the first 4 weeks of treatment. After the mass had been reduced to a diameter of 1 or 2 cm. the rate of regression was much slower. Sometimes this persistent residual nodule would remain unaffected for many weeks. Then, if all injections were stopped, it might disappear within a month. There was a complete failure to reduce the size of the mammary mass in 2 cases. In both instances the size of the breast increased despite treatment over a long period. In at least 2 instances there was a recurrence of the condition, which, however, responded to a second course of treatment. A mass developed in the opposite breast in one patient while the first was mass regressing. This second mass regressed 2 months later, after a series of 16 injections.—From abstract, *J. A. M. A.*

Jacoby, A., D. E. Madonia, S. M. Till and T. H. Wood. The treatment of gonococcal vaginitis by estrogenic hormone. *Am. J. Obst. & Gynec.* 38: 140. 1939.

Treatment of 108 girls in a Social Hygiene Clinic, with 1000-unit amniotin capsules per vagina each night, led to 85% cures, in an average time of 150 days. Relapse occurred in 22%, while no benefit was seen in 15%. There is no constant association of increased vaginal acidity and cures.—E. L. S.

Johnston, J. A. Continuous nitrogen and calcium balances throughout the periods just preceding and following the menarche. *J. Ped.* 13: 937. 1938.

Continuous N and Ca determinations before and after the menarche show a fall in the retention of these substances following the menarche. The basal metabolism throughout this period was irregular, but was relatively elevated before and depressed following the first menstrual period; the retentions of Ca and N were, accordingly, higher with the more elevated rates and lower with the depressed rates.—M. B. G.

Joyet-Lavergne, Ph. Note on the action of male hormone (testosterone propionate) on the masculinization of female organisms (Sur l'action de l'hormone male (propionate de testostérone) dans la masculinisation des organismes femelles). *Compt. rend. Soc. de biol.* 129: 916. 1938.

Living salivary glands of midge larvae were treated with testosterone and then infiltrated with an indicator, cresyl blue or phenosafrinine, to measure the power of oxidation of the cells. It was found that testosterone 1/300,000 had a maximum effect in increasing this oxidation capacity as compared with that of untreated glands. In the author's opinion, the difference between the sexes depends on the greater oxidizing power of the cells in the male. The masculinizing effects of testosterone are, therefore, to be explained on the basis of this property of increasing the oxidizing power of the individual cells.—J. C. D.

Kayser, C., and M. Aron. Cycle of seasonal activity of the endocrine glands in the hibernating hamster (Cycle d'activité saisonniere des glandes endocrines chez un hibernant, le Hamster [Cricetus frumentarius]). *Compt. rend. Soc. de biol.* 129: 225. 1938.

A brief description of the histological appearance of the pituitary, thyroid, adrenals and testes. The glands are in full activity in March and in an involuted state in September.—E. Cutuly.

Kearns, W. M. The clinical application of testosterone. *J. A. M. A.* 112: 2255. 1939.

In 6 castrated patients and in 2 with hypogonadism due to cryptorchidism the administration of testosterone brought about an effective substitution. Beneficial results have been obtained through the intramuscular injection of testosterone propionate and equally good results through the inunction method with free testosterone in a greaseless base.—Author's Summary.

Kellar, R. J., and J. K. Sutherland. Clinical experiences with a new synthetic estrogen stilbestrol (diethyl-stilbestrol): Report to the Therapeutic Trials Committee of the Medical Research Council. *J. Obst. & Gynaec. Brit. Emp.* 46: 1. 1939.

The authors used stilbestrol, a synthetic estrogenic drug, in the treatment of the menopausal syndrome, senile vaginitis, vulval leukoplakia, secondary amenorrhea and for inhibiting lactation. The results were more or less comparable to those obtained with natural estrogen. Therefore it appears that stilbestrol can replace the natural estrogens in all their therapeutic applications, and also it is a drug that is effective by mouth. It apparently has a true localized effect on the genitalia and has presumably the power to depress certain anterior pituitary functions, for example to suppress lactation. In 3 patients in whom uterine bleeding followed a course of stilbestrol, only scanty endometrium was obtained at biopsy. More and more evidence is being produced to show that uterine bleeding is primarily a local vascular phenomenon and is not necessarily directly related to the state of the endometrium. Thus, bleeding can occur from a preovulation or premenstrual or atrophic endometrium. When stilbestrol was given intramuscularly there were no toxic effects. When it was given orally nausea without vomiting was produced in 3 patients but in all these symptoms disappeared even when the use of the drug was continued. From *J. A. M. A.*

Kell, R. C., R. A. Matthews and A. A. Bockman. True hermaphroditism. Report of a confirmed case. *Am. J. M. Sc.* 197: 825. 1939.

The case is reported of an individual who was bisexual in his social attitudes as well as in the possession of well-developed penis and breasts. By assay the sex-hormone output was shown to include estrogens and androgens in low normal amounts. At operation an ovo-testis was found. Upon removal of this the patient seemed to be more masculine, psychologically.—R. G. H.

Klein, M., and G. Mayer. Technic for producing corpora lutea by introduction of luteinizing substance in graafian follicles (Technique permettant la production de corps jaunes par introduction de substance lutéinisante dans les follicules de de Graaf). Production of new corpora lutea in

pregnant rabbits by introduction of luteinizing substance in follicles. Effects on ovarian structure and on the course of pregnancy (Production de nouveaux corps jaunes, chez la lapine gravide, par introduction de substance lutéinisante dans les follicules. Effets sur la structure de l'ovaire gestatif et sur l'évolution de la grossesse). *Compt. rend. Soc. de biol.* 128: 1215; 1218. 1938.

Luteinizing hormone was incorporated in gelatine which was dried and then fragmented. Small slivers were thus obtained which could be inserted through a pin prick made in a follicle of an exposed rabbit ovary. The follicles did not necessarily need to be ripe, and more than one follicle could be utilized. Follicles subjected to the treatment, and only such follicles, became transformed into corpora lutea after 8 days. Control experiments with gelatine slivers not impregnated with luteinizing substance were uniformly negative. Gelatine fragments were inserted into 2 to 5 follicles of ovaries of rabbits pregnant 9 to 13 days, and the animals were autopsied 8 days later. These follicles were luteinized, while the true corpora lutea of pregnancy were undergoing involution. The fetuses were resorbed but the placentae showed little or no change from normal.—E. Cutuly.

LACASSAGNE, A. Mammary adenocarcinoma in male mice treated with a synthetic estrogenic substance (Apparition d'adénocarcinomes mammaires chez de souris mâles traitées par une substance oestrogène synthétique). *Compt. rend. Soc. de biol.* 129: 641. 1938.

Injections of diethylstilbestrol in male and female mice produced effects comparable with those induced by typical estrogens. In male mice subject to cancer this estrogen caused precocious development of mammary carcinoma.—E. Cutuly.

LANCASTER, A. H. Estrogenic hormone therapy in sunlight eruptions of the female. *South. M. J.* 32: 495. 1939.

In 6 cases varying in age from 4 to 36 years, suffering from various forms of photogenic dermatitis, the eruption healed following injections of estrogenic hormone. The older cases showed evidences of menstrual disorders. This suggested that an estrin deficiency may have been a significant factor in the skin condition.—J. C. D.

LANGSTROTH, G. O., AND N. B. TALBOT. The absorption spectra of the compounds formed by androsterone and testosterone in the m-dinitrobenzene reactions. *J. Biol. Chem.* 128: 759. 1939.

The absorption spectra of alcoholic solutions of androsterone and testosterone treated with m-dinitrobenzene and alkali were obtained with the aid of a Spekker photometer and a Hilger E-2 quartz spectrograph. Androsterone gave a maximum at λ5200 and a minimum at λ3800, while testosterone showed a low absorption at λ5200 and a maximum at λ3800 and a minimum at λ3200. Increasing the concentration of dinitrobenzene shifts the absorption of testosterone below λ3000 and obscures the minimum at λ3200. The shape of the extinction curve for androsterone is almost independent of the concentration of the reagents present but that of testosterone is affected by changes in these factors. The reaction involving androsterone is appreciably influenced by the presence of testosterone.—J. M. L.

LAROCHE, G., H. SIMONNET AND E. BOMPARD. Effects of the salts of testosterone on the elimination of gonadotropic principles in the urine (Influence des sels de testostérone sur l'elimination urinaire des principes gonadotropes). *Compt. rend. Soc. de biol.* 129: 953. 1938.

In 18 cases suffering from the effect of the menopause or of castration, testosterone propionate or acetate in sufficient doses had an ameliorating effect on the symptoms and reduced the prolanurea. The action was not as marked or as constant as that of progesterone or folliculin.—J. C. D.

LIPSCHÜTZ, A., AND R. IGLESIAS. Multiple uterine and extragenital tumors produced by estradiol benzoate (Multiples tumeurs utérines et extragénitales provoquées par le benzoate d'oestradiol). *Compt. rend. Soc. de biol.* 129: 519. 1938.

Spayed guinea pigs were injected with 20, 40 or 60γ of estradiol benzoate thrice weekly for periods ranging from 15 days to 4 months. In the majority of animals there developed uterine and extrauterine tumors scattered throughout the abdominal cavity, on the diaphragm, alimentary tract, spleen, etc. Autopsy data are given for 11 animals, and there are numerous photographs.—E. Cutuly.

LIPSCHÜTZ, A., L. VARGAS AND R. IGLESIAS. Structure of uterine and abdominal tumors caused by the action of estradiol benzoate (Sur la structure microscopique des tumeurs utérines et abdominales dues a l'action du benzoate d'oestradiol). *Compt. rend. Soc. de Biol.* 129: 524. 1938.

Prolonged administration of estradiol benzoate to spayed guinea pigs induced the formation of fibrous or fibromyomatous tumors throughout the abdominal cavity (mesentery, uterus, liver, diaphragm, etc.). There was no metastasis to lymph nodes, but there was a tendency on the part of the tumors to invade muscle, pancreas and liver. It was believed possible that coelomic epithelium, lymphatics and vessels participated in the formation of the growths.—E. Cutuly.

McCahey, J. F. Puberty in the male. Cryptorchidism and congenital hypogonadism. *Pennsylvania M. J.* 42: 1179. 1939.

The 2 periods of development in which the adequate function of the testis is essential to establishment of male characteristics are intra-uterine life and adolescence. In the first period the activating agent is the prolan of the placenta; in the second, the exciting factor is the secretion of the basophilic cells of the anterior pituitary. Disturbance of the internal secretory function of the testis usually occurs in the form of hypogonadism. Cryptorchidism is probably the result of testicular hormonal inadequacy during intra-uterine life. Cryptorchidism occurs in 2 types of boys: in the first, the individual is otherwise normal; in the second, there are evidences of hypopituitarism. In the type of cryptorchidism in which the boy is otherwise normal correction of the testicular malposition before puberty is desirable in the interests of normal testicular function. Judicious injections of prolan are suggested as the mainstay in therapy. Untoward results may follow overly prolonged treatment. True congenital hypogonadism, due to hypopituitarism with or without cryptorchidism, may respond slowly to larger doses of prolan. Doses of 200 or 250 R.U. given daily or every other day over long periods of time are suggested. Testosterone propionate may also be tried, but this must be used with caution in children.—I. B.

Macola, B. A., and P. M. Esteve. Variations in blood Ca after hysterectomy with and without ovariectomy (Variaciones de la calcemia en casos de histerectomías con y sin extirpación de ovarios en enfermas con fibromatosis uterina). *Rev. Soc. argent. de Biol.* 14: 195. 1938.

In 16 human cases of hysterectomy and ovariectomy blood Ca increased significantly from 10.29, 0.226 mg.% to 11.66, 0.43 mg.%. In 14 cases in which hysterectomy alone was performed blood Ca decreased from 11.35, 0.287 to 10.73, 0.273 mg.%. The difference between the rise of blood Ca in the first series (8.42%, 3.21%) and the fall in the second (4.95%, 1.65) was 13.37%, 3.60, which has statistical significance.—J. T. L.

McCullagh, E. P. Testicular deficiency due to hypothyroidism. *Cleveland Clin. Quart.* 6: 45. 1939.

A man of 50 complained of impotence gradually increasing for 18 months. The androgen output, by Friedman and capon tests, was notably low. The first diagnosis was functional hypogonadism. Treatment with testosterone at relatively low dosage was begun. There was an increase in androgen output and some symptomatic improvement. After 2 months' treatment the O_2 consumption rate was 68% of prediction. After 6 months the treatment was changed to testosterone propionate 15 mg. per week with improvement of potence. Further increase of dosage was followed by further improvement then by regression when treatment was discontinued. Substitution of desiccated thyroid, 1 grain daily, resulted in as marked improvement as had been obtained with the androgen and in augmented androgen output with thyroid, 3 grains daily, the B.M.R. rose to essentially normal and the patient was practically symptom free.—R. G. H.

McCullagh, E. P., J. M. Rumsey and W. K. Cutler. Urinary excretion of androgens following the injection of testosterone propionate. *Proc. Central Soc. Clin. Research, J.A.M.A.* 112: 1105. 1939.

About 100 assays for urinary androgens are reported for 16 men with hypogonadism and 7 normal men. Single assays have been made in one group following single injections of varying doses, and the ratio of excretion to injection shown. Repeated assays for the same persons appeared to demonstrate diminished rates of excretion as injections continued. Single injections followed by daily assays for hypogonadal persons showed a prompt rise in excretion and a gradual fall to normal. With single doses of from 5 to 10 mg. the excretion of androgen rises to near or to within the lower range of normal, falling to previous levels within one or two days. With larger doses the elevation is much greater. With doses as high as 100 mg., relatively normal levels of excretion may be maintained for about 5 days. Single injections in a normal person caused a prompt rise followed by a distinct depression to below normal and finally a recovery of the original excretion rate in 4 or 5 days. If excreted androgens are calculated as androsterone, it is found that the amount of androgen which can be recovered from hypogonadal persons on the first day following injection of testosterone propionate is from 20 to 50% of the injected quantity, whereas, if assays are made each day for 5 to 7 days, quantities varying approximately from 25 to 75% of the injected substance can be recovered.—Abstract from *J.A.M.A.*

Macgregor, T. N. Dysfunctional uterine bleeding treated with progesterone. *Brit. M. J.* 2: 107. 1938.

Macgregor presents the results of thirteen cases of irregular uterine bleeding, essentially associated with a disturbance of the pituitary-ovarian mechanism, treated with progesterone, 5 mg. given daily. In ovular bleeding the treatment was given 2 or 3 days before the expected anomalous bleeding, the aim being to maintain endometrial stimulation during the time of ovulation and thus tide over the period when the estrogenic content of the blood was lowered. In hemorrhagic metropathia progesterone may be given at any phase. During the bleeding stage 5 mg. was administered daily until the bleeding was controlled. During the amenorrheic phase 5 mg. was given daily, the total dosage amounting to from 25

to 30 mg. Three of the 5 patients with the ovular type of bleeding have had regular menstruation for the last 18 to 22 months. One patient had 3 regular periods after cessation of therapy: she then became pregnant and aborted at the end of the 2nd month of gestation. The other patient, though not completely well, has now only a brownish discharge, whereas formerly she had frank hemorrhage for a few days between every two periods. In 4 of the 7 cases of anovular bleeding menstruation became normal in duration and periodicity, in 2 of which there has recently been a recurrence of the irregularity. In the remaining 3 patients the uterine endometrium was examined microscopically before and after treatment and as a result of the treatment the hyperplastic endometrium has been changed into a pseudosecretory phase. These patients have only recently been under treatment and 2 have had 2 normal periods. Comparatively few favorable results follow the indiscriminate use of progesterone therapy.—From abstract in *J. A. M. A.*

MANNHEIMER, E. Pubertas praecox due to dextrolateral granulosa cell tumor of the ovary in a 4-year-old girl. *J. Ped.* 12: 351. 1938.

The patient, a 4-year-old girl, showed in addition to menstruation, increased growth in stature, advanced ossification and appearance of secondary sex characters. The Aschheim-Zondek reaction was negative. Prolan test showed 9 mouse units per liter of urine, an insignificant amount. Folliculin was present in the tumor as demonstrated by implantation into mice. The removal of a granulosa cell tumor of the ovary was followed by an improvement in psyche, cessation of menstration and slowing down of general growth and ossification. According to Gland and Goldstein, in 1935, only 7 of about 250 published cases of granulosa-cell tumor of the ovary have occurred in children under 10 years of age. In some cases there is an increased quantity of folliculin in the urine and blood of the patient and a quantity of prolan that is normally encountered only in pregnancy, and a positive Ashheim-Zondek test.—M. B. G.

MARAINI, B., AND A. TRABUCCO. Effect of testosterone propionate on prostate adenoma in human beings (Efectos clínicos de las inyecciones de propionato de testosterona en el adenoma de la prostata). *Bol. Acad. Nac. Med. Buenos Aires.* 832. 1938.

In 19 cases improvement in the general condition was found; subjective symptoms and frequency diminished; small increases in the prostate of recent standing were considerably bettered; in those of longer standing little or no effect was obtained. Prolonged treatment was necessary to obtain results. The dose varied with the individual, for each a maximum effect was obtained and larger doses did not increase it.—J. T. L.

ESTEVE, P. M. Effect of ovarian hormones and gonadotropic substances in urine on the genital tract of *Didelphys azarae* (Acción de las hormones ováricas y de los principios hipofisoides de la orina sobre el tractus genital del marsupial *Didelphys azarae*). *Rev. Soc. Argent. de Biol.* 14: 509. 1938.

The gonadotropic substances of pregnancy urine and of urine from patients with a seminoma produced follicular maturation and luteinization in the ovary and typical progestational changes. In ovariectomized animals these changes were not observed. Moderate doses of estrone (20,000 U in 25 days) produced a marked development in the genital tract of females, without modifying the ovary. Progestational changes were only seen when subsequently progesterone was administered.—J. T. L.

MARTINS, T. Contractility and pharmacology of the prostate of normal and castrated rats and castrated rats injected with sex hormones (Contractilité et pharmacologie comparee *in vitro*, de la prostate de rats normaux et de rats castrés, et injectés d'hormones sexuelles). *Compt. rend. Soc. de biol.* 129: 71. 1938.

Contractility of rat coagulating glands in Locke solution was determined with a myograph. Glands were obtained from 12 normal rats, 14 rats castrated 11 to 125 days, and from castrated rats injected for varying periods with various male and female sex hormones or drugs. As a rule motility was exhibited by glands from all of the animals except the normal ones and the castrates injected with testosterone.—E. Cutuly.

MARX, W., AND H. SOBOTKA. Colorimetric determination of equilenin and dihydroequilenin. *J. Biol. Chem.* 124: 693. 1938.

A method is described in which equilenin and dihydroequilenin are extracted from mare's urine with ether or chloroform and then coupled with p-nitrobenzene azodimetoxyaniline in 55% alcoholic solution. The insoluble product is removed by centrifugation and dissolved in benzol. The resulting blue solution is read in the colorimeter against a known equilenin standard. The coupling does not take place with estrone, estriol estradiol, equilin or dihydroequilin.—J. M. L.

MASON, L. W., AND R. G. GUSTAVSON. The quantitative determination of estrogenic substances in normal female urine through the inception of a pregnancy. *Am. J. Obst. & Gynec.* 36: 1026. 1938.

The graphic record shows the estrin secretion for 45 days, beginning before the ovulation which

was followed by conception. Marked fluctuations occur from day to day, with peaks suspected of marking ovulation and the onset of function of the corpus luteum of pregnancy.—E. L. S.

MAYER, A. Menstrual bleeding and trauma (Menstruationsblutungen und Trauma). *Zentralbl. f. Gynäk.* 62: 2578. 1938.

In addition to theoretical lists of possible mechanisms, the author cites three cases of concussion with periods of amenorrhea and irregularities of cycle. No autopsy data.—E. L. S.

MAZER, C. Puberty in the female. *Pennsylvania M. J.* 42: 1176. 1939.

This is a summary of the normal and morbid phenomena manifested by the female during the puberty period. The first menstruation is but one of the many manifestations of puberty and connotes the beginning stabilization of the pituitary-ovarian complex and the intrinsic capacity of the uterus to respond to adequate hormonal stimulation. Mazer finds that the first menstrual flow usually occurs at the age of 13.2 years. Its absence at 16 portends future menstrual irregularities and its nonappearance at 18 indicates a severe degree of endocrine or metabolic disturbance. The length of the normal cycle is from 21 to 35 days. Delayed inception of menstruation is due to inherent pituitary, ovarian or endometrial inactivity, with or without such nonendocrine factors as anemia, tuberculosis and undernutrition. Lack of uterine response to a normal pituitary-ovarian mechanism can be corrected by large doses of estrogen given every third day for 2 weeks of the month. Intractable menorrhagia or metrorrhagia during puberty may yield to thyroid therapy or to insulin if the third is undernourished. In severe cases of uterine bleeding low dosage irradiation of the pituitary and ovaries may be required, or as a last resort injections of testosterone propionate. The etiological and therapeutic aspects of abnormalities of growth, such as pituitary gigantism, eunuchoid overgrowth and dwarfism are briefly discussed.—I. B.

MAZER, C., AND G. BAER. The therapeutic value of low-dosage irradiation of the pituitary gland and ovaries in functional menstrual disorders and sterility. *Am. K. Obst. & Gynec.* 37: 1015. 1939.

The authors give 3 weekly doses, which allows what they estimate as about 0.1 of a skin erythema dose to reach the ovaries, with similar dose applied to the pituitary. There are 178 women, of whom 106 had amenorrhea, 26 menorrhagia, 18 metrorrhagia, and 3 normal rhythm with scanty flow. Twenty-five others had normal rhythm, with other anomalies. The beneficial results appeared to be greater in proportion to the lesser deviation from normal function, with marked improvement reported. Pregnancies followed in a number of patients. Only one case seemed to have any harmful results. The authors consider this as stimulative therapy, although they adduce no direct evidence of stimulation.—E. L. S.

MENGERT, W. F. Precocious puberty due to an ovarian cyst in a 5-year-old girl. *Am. J. Obst. & Gynec.* 37: 485. 1939.

An illustrated case report of an acutely developed single cyst, with 2-year cure by laparotomy. Urine and cyst fluid estrogen assays were high, dropping to no estrogen in urine. The tumor was not malignant. Puberal symptoms regressed.—E. L. S.

MOORE, R. A., AND A. M. MCLELLAN. A histological study of the effect of the sex hormones on the human prostate. *J. Urology* 40: 641. 1938.

In 10 consecutive cases of prostatic enlargement, 5 were given injections of androgenic hormone. The others received injections of estrogenic hormone. Careful histological examinations of the removed glands were then made. The authors summarize the observations. "The injection of from 285 to 1,125 mg. of testosterone propionate in from 12 to 95 days results in no significant restoration of the involuted prostate of presenility and in no observable alteration in the histological appearance of the tissues formed in benign hypertrophy. The injection of from 15,000 to 140,000 I.U. of estradiol benzoate in from 10 to 31 days produced conspicuous alteration in the urethral and ductral epithelium but little if any change in the tissues of benign hypertrophy."—J. C. D.

MORICARD, R., AND J. CAUCHOIX. Production of large fibromata in female guinea pigs by injection of dihydrofolliculin benzoate (Réalisation de volumineux fibromes chez la femelle de cobaye par l'injection de benzoate de dihydrofolliculine). *Compt. rend. Soc. de biol.* 129: 556. 1938.

Thirteen female guinea pigs were injected weekly for periods from 8 to 32 weeks with doses of dihydrofolliculin totalling 8 to 160 mg. Uterine and extrauterine fibromata were of frequent occurrence in these animals. It was possible to localize the tumors around foreign bodies (thread) in the uterus.—E. Cutuly.

MURRAY, E. G. Studies on changes in cells of vagina (Studien über Veränderungen des Zellinhaltes der Vagina). *Arch. f. Gynäk.* 165: 635. 1938.

The author divides the epithelium of the vagina into 5 layers; germinative, stratum spinosum composed of deep cells and upper cells, layer of karyopyknotic cells, stratum granulosum (granular cell

layer), and horny layer. The germinative cells cannot be recognized in vaginal smears, but the cells from the other 4 can be and the two types of cells from the stratum spinosum can also be differentiated. The method of study consisted in determining at frequent intervals the proportion of each of the 5 types of cells in vaginal smears. Studies included one case with a 28-day cycle, one case with a 21-day cycle, various types of ovarian dysfunction both before and during estrin therapy, and a case of pregnancy. The conclusions are too numerous to list. The final conclusion, however, is that whereas the method is of value for investigative purposes, "its use in practice is limited because of the difficulties in the interpretation of the findings and the diversity of the results."—F. A.

Muschat, M., M. Labess and D. Meranze. Gonadal activity in prostatic hypertrophy. *J. Urology* 40: 805. 1938.

After studying normals and 30 cases of prostatic hypertrophy in 10 of which it was possible to make careful assays on 24-hour samples, the authors conclude: "The creatinine utilization test for male sex hormone was used in determining the gonadal activity in the male at varied periods of maturity. The normal adult male shows creatinine retention below 13%. The eunuch and eunuchoid show marked creatinine retention 35 to 50%. There is a noted decline in the activity of the gonads after the age of 50. The cases with prostatic hypertrophy have the same creatinine retention as the normal male adults, indicating continued gonadal activity. The hypothesis that prostatic enlargement is caused by abnormal continued gonadal activity after the age of 50 is thus advanced."—J. C. D.

Nagel, M., and H. Bachmann, Management of primary weak labor contractions with follicle hormone (Die Behandlung der primären Wehenschwäche mit Follikelhormon). *Zentrlbl. f. Gynäk.* 63: 598. 1939.

Since the physiological studies suggest that the effect of estrogenic substances is to increase the muscular activity and the sensitivity of uterine muscle, of progesterone to reduce this activity and sensitivity, and since there is a decrease of progesterone production toward term, combined with an increase of estrogen, it seems worth while to use estrogens to increase the sensitivity and force of uterine muscle. The authors report 14 cases treated at term with estradiol benzoate in oil, 30,000 to 250,000 U, in which labor followed in a very few hours. In some cases quinine, posterior pituitary, or thymophysin or some combination of these were used also to initiate activity. Sometimes these latter methods failed when tried alone, until repeated after the use of the estrogen. The authors believe the short time interval between injection and success is due to the greater effectiveness of estradiol as compared with estrone preparations, previously reported by others. This prompt success also makes intravenous therapy unnecessary. There were no unfortunate sequellae. In two cases Cesarian section gave visible evidence of the increased muscular activity induced by the therapy. Postpartum bleeding was favorably influenced. —E. L. S.

Nelson, A. A. Giant interstitial cells and extraparenchymal interstitial cells of the human testis. *Am. J. Path.* 14: 831. 1938.

Microscopic sections of 721 testes from 470 autopsies of individuals 18 years of age or more were studied. Giant interstitial (Leydig) cells having from 4 to 30 (usually 8 or 10) nuclei were found in 85 testes. They were found at all ages and in all sorts of general disease conditions. They do not appear to have been described previously. The observations of others on the 'sympathico-tropic' or 'hilus' cells have been confirmed and extended. These cells in the testis are held to be identical with the ordinary interstitial or Leydig cells. The author has proposed the name 'extraparenchymal interstitial cells' or 'extraparenchymal Leydig cells' as best describing them.—W. J. A.

Nixon, N. The undescended testicle. *Am. J. Dis. Child.* 55: 1037. 1938.

The use of endocrine therapy is justifiable in the treatment of cryptorchidism. The author reviews reports in the literature of 388 boys with 574 undescended testes who were treated with intra-muscular injections of various gonadotropic substances and adds 59 cases of his own. The percentage of successful results range from 100 to 19% with an average of 59%. Approximately one-third more of the patients with bilateral retention responded to treatment than those with unilateral conditions. The author obtained descent in 25% in his personal series following the intra-muscular injection of 250 R.U. of pregnancy urine extract three times each week until 30 injections had been given, a total of 7,500 R.U. A three months' rest was given if the testes had not descended by this time. Operation was done on 17 of 37 patients whose testes failed to descend completely after treatment. An atrophied testis was found in 2 cases, there was an apparent congenital absence of the testes in 3 cases. In only 7 cases could the orchidopexy be considered successful as based on observations several months later.—M. B. G.

Noble, R. L. Functional impairment of the anterior pituitary gland produced by the synthetic oestrogenic substance 4:4′ dihydroxy:β-diethylstilbene. *J. Physiol.* 94: 177. 1938.

Nine ♂ rats were injected with total doses of 10 to 30 mg. of diethylstilbestrol over a period of

from 5 to 28 days. Seminal vesicles and prostate were much reduced in weight, but testes were sometimes reduced and at other times unchanged. The pituitary gland was enlarged. It is assumed that since the organ weights achieved with the estrogenic substance were similar to those of an hypophysectomized rat, the gonadotropic activity of the hypophysis had been completely suppressed. Nine ♀ rats similarly treated showed ovarian weights comparable to those of hypophysectomized animals. The pituitary glands and the adrenals of both ♂ and ♀ were enlarged, thereby differing sharply from the hypophysectomized condition. Massive doses (200 mg. in 20 days) caused ovarian atrophy in ♀'s examined 7 weeks after last injection, but in ♀'s one week after the injections had ceased the ovaries were normal or were greatly hypertrophied. The pituitaries and adrenals also showed large weight increases directly after treatment but a gradual decrease in weight to the 7th week. The figures are somewhat exaggerated inasmuch as all weights were expressed in terms of weight per 100 gm. of body weight. During the severe injections and at the close, the animals actually showed heavy weight losses, but at the end of 7 weeks showed a gain in body wt. After treatment with this synthetic estrogen ♀ rats responded with an increase in ovarian weight to pregnant mare serum and PU extract. A study of water balance changes indicated that no alteration in the elaboration of the diuretic principle by the anterior lobe had occurred.—A. E. Severinghaus.

Novak, E. Clinical syndromes referable to failure of ovulation. *Am. J. Obst. & Gynec.* 37: 605. 1939.

In a summary the author presents his conviction based on his own and other published experiences that anovulatory flowing is far commoner than usually believed, especially in the early and late years of the menstruating period. This is due to hypofunction of the ovaries, and presumably to under-stimulation by the pituitary. Therapy for this condition is still unsatisfactory, even with intravenous use of pregnant mare serum concentrates. Such anovulatory flows may occur in regular or irregular cycles, with little or excessive flow, and can only be diagnosed by endometrial tissue examination shortly before the expected flow.—E. L. S.

Odell, A. D., and G. F. Marrian. Some observations on the constitution of the 'pregnanetriol' occurring in the urine of pregnant mares. *J. Biol. Chem.* 125: 333. 1938.

The constitution of a saturated solid alcohol present in the urine of pregnant mares was studied. Partial hydrolysis of the acetate yielded a product melting at 222–224°, which was thought to be the dihydroxymonoacetate of the parent triol $C_{21}H_{36}O_3$. On oxidation with chromic anhydride the corresponding diketo product was obtained; it melted at 191–192°; it was shown by the formation of a pyridazine derivative to be a γ-diketone. By reduction of the diketo monoacetate by the Wolff-Kishner method, a monoatomic alcohol of m.p. 82–83° was obtained. It was suggested that the substance was a pregnane. or allopregnane 3(α) 6,20 triol and not pregnane 3(α) 4,20 triol as suggested by Marker.—J. M. L.

Offenkrantz, F. M., and A. M. Staniel. Serum cholesterol fluctuations during the menstrual cycle. *Am. J. Clin. Path.* 8: 536. 1938.

Offenkrantz presents data for each of a total of 16 monthly cycles in 16 young, healthy and regularly menstruating women. About 8 determinations in a cycle were performed. The following facts were observed: 1. There is a sharp elevation of the total serum cholesterol at the end of the bleeding phase, associated with a slightly lowered percentage of free cholesterol. 2. There is a lowered total serum cholesterol at the onset of the menses. The percentage of free cholesterol is higher at this time than at its termination. 3. The intermediary cholesterol levels show a tendency to rise to the middle of the cycle and then fall off toward the new cycle. 4. Although there were wide fluctuations for each subject, no determination was outside the values previously found in other series on normal subjects. 5. There appear to be definite planes (i.e., higher, lower or intermediate levels) along which all the total cholesterol values for any subject will vary. These planes are held to be determined, in part, by the constitutional reaction type of the subject. 6. The narrow limits of fluctuation of the percentage of free cholesterol indicate the importance and stability of this factor. This variation is from 22.09 to 32.9% of the total cholesterol. The meaning of this variation of cholesterol values with regard to the menstrual cycle is discussed.

Palmer, A. Excretion of hormones in a case of habitual abortion. *Am. J. Obst. & Gynec.* 36: 1005. 1938.

Urinary estrin and gonadotropic concentrations were recorded at weekly intervals for ten months terminated by another abortion. The peak of gonadotropic hormone was passed after 2 months from conception, and there was a marked rise in free estrin before the abortion at the 14th week.—E. L. S.

Palmer, A. Estrogenic hormone in the urine and tumor of a patient with a granulosa cell tumor of the ovary. *Am. J. Obst. & Gynec.* 37: 492. 1939.

A marked increase in estrin excretion by this 19-year-old woman was reduced to ordinary excretion levels after removal of the tumor, which contained 20,000 I.U. of estrone equivalent per kg. of tumor removed.—E. L. S.

PARKES, A. S., E. C. DODDS AND R. L. NOBLE. Interruption of early pregnancy by means of orally active oestrogens. *Brit. M. J.* 2: 557. 1938.

The capacity of the highly active estrogens, ethinyl estradiol and diethyl-stilbestrol, to inhibit progestational changes in the uterus has been followed after oral administration. Both of these substances were shown to inhibit the effect of progesterone and prevent implantation of the blastocysts in rabbits. Small doses of diethyl-stilbestrol prevented implantation in rats, while ethinyl estradiol was highly effective in interrupting established pregnancy in rabbits.—H. J. D., Jr.

PARKS, J. Granulosa cell tumors of the ovary with precocious puberty. *Am. J. Obst. & Gynec.* 36: 674. 1938.

An illustrated case report of a 5-year-old girl shows how the precocious menstruation associated with advanced skeletal growth may be cured by removal of a granulosa cell tumor from one ovary. From a series of 45 ovarian malignant tumors, there were only 5 of this type, but all the others were in adults, and all were unilateral.—E. L. S.

PATERSON, S. J. The therapeutic uses of progesterone. *Edinburgh M. J.*, 46: Trans. Edinburgh Obs. Soc. 49. 1939.

The author reviews our present knowledge and then considers 38 cases of eclampsia and pre-eclamptic conditions in which progesterone was used. Both these groups showed a better mortality rate for mother and child than groups of similar cases where progesterone was not used. If the progesterone treatment is regarded as a substitution therapy, the necessity for its early application, as well as for the ordinary therapeutic measures called for by the patient's condition, will be appreciated. The author regards the corpus luteum deficiency as the basic condition in eclampsia. He suggests that a study of the pregnandiol output might give early warning of such failure.—J. C. D.

PEVAROFF, H. H., AND L. H. BISKIND. The pregniotin test for pregnancy. *Am. J. Obst. & Gynec.* 38: 315. 1939.

The use of pregniotin, an antigen from placenta, for intracutaneous testing to diagnose pregnancy gave positive tests in 21 of 22 pregnant women, continued to do so for 1 to 12 days post partum, but gave 2 false positive tests in 6 males, and in 10 non-pregnant females.—E. L. S.

HYPOPHYSIS

BATES, R. W., O. RIDDLE AND E. L. LAHR. The racial factor in the pigeon crop-sac method of bioassay of prolactin. *Am. J. Physiol.* 125: 722. 1939.

Different races and strains of doves and pigeons may differ markedly in the response in their crop sacs to prolactin. These variations in response were more extreme when high dosage of prolactin was employed. When any race of pigeons is used for quantitative assay of prolactin by the weight method it is first necessary to determine the useful range over which crop-sac weight increases with dosage, and thereafter to establish a racial correction factor with the aid of a standard prolactin preparation.—From the Authors' Summary.

BENDA, C. E. Studies in mongolism. III. The pituitary body. *Arch. Neurol. & Psychiat.* 42: 1. 1939.

The histologic pictures in 13 cases of mongolism and in 1 case of premature birth are presented. In the 13 cases of mongolism definite alterations peculiar to this condition were invariably observed. The alterations consist in an increase of eosinophilic cells and a deficiency of basophilic and chief cells. In analyzing these alterations, the question is discussed whether the increase of eosinophilic cells or the absence of basophilic cells has the greater pathologic significance. Because the size of the pituitary is not increased and no signs of eosinophilic hyperactivity are found, the absence or deficiency of basophilic cells appears to be the most important observation. This deficiency seems to be characteristic of and definitely associated with the clinical picture known as 'mongoloid deficiency.' In discussing the significance of the observations, the difference between morphogenetic and morphokinetic factors is emphasized. There are many indications that mongolism is the result of a morphokinetic failure and is due to an endocrine disturbance. The key to the general disorder seems to be a particular pituitary deficiency, which may be due to a pituitary deficiency of the mother during pregnancy. The advanced maternal age, frequently observed in cases of mongolism, and other factors indicate that the maternal organism is not apt to adjust itself to a new pregnancy.—Author's Summary.

BOYD, E. M., AND D. W. WHYTE. The effect of posterior hypophyseal extract on the retention of water and salt injected into frogs. *Am. J. Physiol.* 125: 415. 1939.

Previous work has shown that pituitrin inhibits the loss of normal body water from frogs. Here it is demonstrated that the water diuresis inhibiting action of posterior pituitary extracts also applies to

extra (added) body water. Evidence that water retention potency may be fractionated in the order pitocin, pituitrin, pitressin is presented. Sodium chloride addition can counterbalance and reverse the retention potency. This salt effect is augmented by a 'salt mobilizing' action of posterior pituitary extracts, pitressin showing the greatest potency.—H. J. Spoor.

Carrière, G., J. Morel and P. J. Gineste. Influence of anterior lobe extract on the thymus of thyroidectomized rats (Influence de l'extrait de lobe antérieur d'hypophyse sur la morphologie du thymus chez le rat thyréoprive). *Compt. rend. Soc. de biol.* 128: 1151. 1938.

AP extract administered daily for 56 days was incapable of stimulating growth of the thymus gland in rats thyroidectomized when 2 months old. In normal rats the extract caused hypertrophy of the thymus. These data considered in the light of the fact that involution of the thymus occurs after thyroidectomy led to the conclusion that stimulation of the thymus is mediated through the thyroid.—E. Cutuly.

Crandall, L. A., Jr. and I. S. Cherry. The effects of insulin and glycine on hepatic glucose output in normal, hypophysectomized, adrenal denervated and adrenalectomized dogs. *Am. J. Physiol.* 125: 658. 1939.

The effects of insulin (subcutaneous) and glycine (oral) on glucose and lactic acid in the portal, hepatic and arterial blood were studied by the angiostomy technic on normal, hypophysectomized, adrenal denervated and adrenalectomized dogs. The significance of results was determined by statistical analysis. In normal dogs there was a positive correlation between fasting arterial blood sugar and hepatic glucose output and a negative correlation between fasting hepatic output and change in hepatic glucose output after insulin. Twenty grams of glycine caused the arterial blood sugar to rise and produced a 100% increase in hepatic glucose output. All three types of operated animals showed insulin shock about 1 hr. after subcutaneous injection of 15 U of insulin. In hypophysectomized dogs the arterial blood sugar response to insulin parallels that of normal dogs but occurs at a lower level. The fasting hepatic glucose output of such animals was less than 50% of the value for normals and did not increase during the hypoglycemic stage of insulin action. The effect of glycine was similar to that in normal dogs. In adrenal denervated dogs the fasting arterial blood sugar was within normal limits but the drop following insulin was more abrupt and the level after one hour was below normal. The fasting hepatic glucose output was lower than in normal animals but not significantly so. The changes following insulin were similar to those in hypophysectomized animals. Adrenalectomized dogs maintained on high sodium low potassium diets reacted in all experiments similar to adrenal denervated animals. In none of the experiments were there any significant changes in blood lactic acid.—F. A. Hitchcock.

Corcoran, A. C., and I. H. Page. The effects of renin, pitressin, and pitressin and atropine on renal blood flow and clearance. *Am. J. Physiol.* 126: 354. 1939.

The slow infusion of pitressin may result in increased or decreased renal blood flow without definite effects on arterial pressure during the experimental period. The absence of increased arterial pressure in the presence of decreased renal blood flow and efferent arteriolar constriction due to pitressin does not exclude the possibility of a secondary renin action during prolonged infusions, since the onset of experimental renal hypertension due to constriction of the renal artery is usually delayed for 24 hours or more. Increased arterial pressure apparently of cardiac origin may be produced by the injection of atropine during the infusion of pitressin. The arterial hypertension results in increased glomerular filtration but has no characteristic effect on renal blood flow.—From the Authors' Summary.

Foley, M. P., A. M. Snell and W. M. Craig. Anterior pituitary tumor associated with cachexia, hypoglycemia and duodenal ulcer. *Am. J. M. Sc.* 198: 1. 1939.

The presenting symptoms of a patient with a large cystic chromophobe adenoma of the anterior hypophysis were those of penetrating duodenal ulcer. Such a lesion was demonstrated roentgenologically and symptomatic relief was obtained on ulcer management. The patient also had definite hypoglycemia; the level of the blood sugar was not permanently elevated by a high carbohydrate diet, intravenous infusions of glucose, suprarenal cortical hormone, or anterior pituitary extracts. Epinephrine produced a slight temporary increase. The blood sugar was not affected by removal of a portion of the tumor and subsequent radiotherapy. Cachexia, eunuchoidism and lowered metabolism were additional features. The probable relation of these symptoms to the original hypophyseal lesion is discussed.—Authors' Summary.

Friedgood, H. B. Induction of estrous behavior in anestrous cats with the follicle-stimulating and luteinizing hormones of the anterior pituitary gland. *Am. J. Physiol.* 126: 229. 1939.

Six young adult anestrous cats were injected daily with a highly purified extract of the anterior pituitary gland containing follicle-stimulating hormone (FSH) with traces of the luteinizing hormone (LH). Five of the cats receiving a total of 22 to 84 R.U. of FSH came into heat within 7 to 13 days. The

behavior of these cats before, during and after mating was identical with that of cats in spontaneous heat and their ovaries contained normal estrous follicles. The 6th cat, which received 109 FSH units for 6 days did not come into heat; autopsy showed it to be a surgical castrate. The hormone preparation is believed to have acted by means of ovarian stimulation.—S. L. Cohen.

Goldberg, I. Hypophysectomy and plasma proteins (Action de l'insuffisance hypophysaire sur les protéines du plasma sanguin). *Compt. rend. Soc. de biol.* 128: 1135. 1938.

Hypophysectomy in dogs after 21 to 358 days caused increase in plasma globulins and N.P.N. and a decrease in albumins. Thyroid feeding tended to correct these changes. In toads hypophysectomy did not have the same effects as in dogs.—E. Cutuly.

Haterius, H. O., and J. K. W. Ferguson. Evidence for the hormonal nature of the oxytocic principle of the hypophysis. *Am. J. Physiol.* 124: 314. 1938.

In 12 rabbits, 2 to 80 hours post partum, electrical stimulation in the region of the infundibular stalk produced a definite increase in uterine activity, characterized by increased frequency and amplitude of contractions. This response resembled closely that obtained by injection of pitocin and differed radically from the effects of epinephrine. The response persisted after spinal transection, after section of the splanchnic nerves and after vagotomy, but was effectively blocked by electrolytic lesions placed directly in the stalk of the pituitary body. On the basis of these observations it is believed that the pituitary complex secretes the oxytocic principle and that the latter need no longer be considered as merely a pharmacological principle but may properly be classed as a hormone of the pituitary gland.—Authors' Summary.

Hayward, S. J., J. H. Pollock and L. Loeb. The effects of formalin on the thyroid stimulating and gonadotropic hormones of cattle anterior pituitary glands. *Am. J. Physiol.* 125: 113. 1939.

Atresin, luteinization and thyroid stimulating effects of anterior pituitary glands were suppressed by exposure of the glands to formalin. Glands so treated produced heat changes in the female sex organs while normal glands produce very slight effect. The pH of the formalin solution was significant, also the season during which the experiment was performed was important.—F. A. H.

Hsieh, W. M. A vagus-post-pituitary reflex. The glucogenic effect. *Chinese J. Physiol.* 13: 187. 1938.

Faradic stimulation of the afferent vagus or the peripheral cervical sympathetic in cats produced a transient moderate increase of the blood sugar concentration up to 50 mg.%. The hyperglycemia was obtainable after crushing of the neuraxis but not after hypophysectomy. The mechanism of the hyperglycemia is discussed. It is suggested that vagal hyperglycemia is mediated by the posterior lobe, whereas that of the sympathetic probably involves the anterior lobe of the pituitary gland.—Author's Abstract.

Ingle, D. J., H. D. Moon and H. M. Evans. Work performance of hypophysectomized rats treated with anterior pituitary extracts. *Am. J. Physiol.* 123: 620. 1938.

The work performance of hypophysectomized rats is greatly decreased. The work performance of hypophysectomized rats treated with growth, mammotropic and thyrotropic extracts is definitely improved. Hypophysectomized rats treated with adrenocorticotropic hormone show a greater improvement in work capacity than those treated with other pituitary extracts, and compare favorably with those treated with cortin. The work capacity of hypophysectomized, adrenalectomized rats maintained with cortin is not improved by the administration of adrenocorticotropic extracts. The work capacity of hypophysectomized rats treated with any of the AP extracts is less than normal.—Authors' Summary.

Jones, H. M. Possible anatomic relations between pituitary and prostate gland. I. Normal pituitary body. *J. Urology.* 41: 84. 1939.

After determining that the pituitary glands were within the limits of normal, these glands and the prostates from 168 autopsies were thoroughly examined. The latter were normal in 110 cases, atropic in 7, and enlarged in 51. The authors found that: "Wide variations in the microscopic structure of the normal pituitary body may be observed at any age. These variations involve the cellular types found in the anterior lobe of the pituitary body, the acini of the pars intermedia and the basophilic infiltration of the pars nervosa." Variations in these three factors could not be correlated with changes in the size or microscopic structure of the prostate gland.—J. C. D.

Kleiber, M., and H. H. Cole. Body size and energy metabolism in growth hormone rats. *Am. J. Physiol.* 125: 747. 1939.

The growth hormone of the pituitary was injected into 22 female rats daily for 6 months. The thyroids and adrenals were found to be small in these animals. On the basis of weight or surface area the metabolism measured by CO_2 production and O_2 consumption was reduced by these injections. The in vitro metabolism of the diaphragm was also lowered. The authors conclude that there is somatic adaptation of tissue metabolism in vivo and in vitro as a result of changes in the endocrine system.—G. C. Ring.

KLOTZ, R. Pituitary insufficiency in and after pregnancy, a contribution to the problem of birth accidents (Hypophyseninsuffizienz in und nach Schwangerschaft, ein Beitrag zur Problem der Fehlgeburt). *Zentralbl. f. Gynäk.* 63: 1844. 1939.

After a discussion of the symptoms of Simmonds' cachexia the author asserts that a syndrome of hypotension, psychasthenia, gastric atony and atony of other smooth muscle is typical of pituitary insufficiency. Cases are reported with menorrhagia, threatened abortion, emesis of pregnancy and uterine atony, in which striking improvement followed the use of präphyson by injection and by tablets. The exact doses are not clearly stated. The cases include both acute and chronic syndromes, but all those reported recovered and did not require continuous therapy.—E. L. S.

LARSON, E. Fate of the oxytocic principle of the posterior pituitary. *J. Pharmacol. & Exper. Therap.* 66: 22. 1939.

Injecting 3 or 8 mg. doses of posterior pituitary into cats, resulted in elimination of about 27% of the oxytocic principle in the urine. Dogs eliminated a lesser amount. Tissue polypeptidases inactivate the oxytocic principle.—E. L.

LASSEN, H. C. A., AND L. HANSEN. Investigations into the effect of anterior pituitary extract on the carbohydrate metabolism in normals and diabetics. *Acta. Med. Scandinav. Supplement* 89: 288. 1938.

The injection of an alkaline pituitary extract in dosages equivalent to 2.5 gm. of fresh tissue caused a pronounced diabetogenic effect in 2 normal and 3 diabetic subjects. There resulted a marked increase in ammonia excretion, presumably as a result of the ketogenic action of the extract. The effect is best seen after meals containing a moderate amount of protein and carbohydrate. If amounts of carbohydrate of the order of 75 gm. are given, the extract does not elevate the peak of the sugar curve but does increase the length of time during which the blood sugar remains elevated. The extract may cause a temporary lowering of the renal sugar threshold in normal healthy persons.—J. M. L.

LAUSON, H. D., J. B. GOLDEN AND E. L. SEVRINGHAUS. The gonadotropic content of the hypophysis throughout the life cycle of the normal female rat. *Am. J. Physiol.* 125: 396. 1939.

The gonadotropic content of the normal female rat hypophysis in infancy, pre-puberty, puberty: adult sexual life and senility has been investigated. Pituitaries from 248 donors, ranging from 14 days to 2½ years of age, were assayed by the uterine method on 272 immature test rats, 22 days old. All adult rats were killed in proestrus. The pituitary potency rises to a peak at 21 days and decreases gradually until the onset of puberty. At puberty, there is a rapid drop in potency to about one-half of the prepuberal level. Throughout adult sexual life, the content of the hypophysis in the proestrous stage remains constantly at the low puberal level, despite continued increase in pituitary size. This is interpreted to mean that the adult gland stores very little of its gonad-stimulating complex, but releases it as rapidly as formed. Limited study of the senile age has revealed that the potency markedly increases as the ovaries fail. The assumed parallel between the natural and artificial climacteric seems reasonably established.—Authors' Summary.

LAMBILLON, J., AND A. LEJEUNE. Can melanophore expansion in frogs serve as a test for posterior pituitary activity? (La reaction d'expansion des chromatophores de la grenouille peut-elle servir de test fonctionnel post-hypophysaire?) *Compt. rend. Soc. de biol.* 128: 1138. 1938.

Expansion of melanophores in frogs injected with human urine has been reported to be a specific test for hyperactivity of the posterior lobe of the pituitary. Evidence is presented to show that the reaction is non-specific.—E. Cutuly.

MACKAY, E. M., AND A. N. WICK. Influence of adrenalectomy on the blood and urine ketones during fasting and anterior pituitary extract administration. *Am. J. Physiol.* 126: 753. 1939.

Using 150 rats, the authors determined the effect of fasting, of fasting+adrenalectomy and of fasting+adrenalectomy+anterior pituitary extract injections on ketonemia and ketonuria. Urine ketones were determined by the method of Van Slyke and blood ketones by that of Barnes. Adrenalectomy led to the rise in the renal threshold for ketone bodies so that the urine might be almost entirely free of these compounds while the blood ketone levels were very high. Assuming a reduction in blood ketone level to be due to less ketogenesis and not increased ketone body oxidation the blood ketone levels after adrenalectomy indicated only a slight lowering of ketone production during fasting after the removal of the glands.—K. A. Brownell.

MAKEPEACE, A. W. The effect of progestin upon the anterior pituitary. *Am. J. Obst. & Gynec.* 37:457. 1939.

This very brief review of papers by the author and others presents evidence that the failure of rabbits to ovulate during pregnancy or pseudopregnancy is due to the presence of a functioning corpus luteum, the progestin from which inhibits the liberation from the anterior pituitary of gonadotropic material in sufficient amount to cause ovulation.—E. L. S.

MARTIN, S. J., H. C. HERRLICK AND J. F. FAZEKAS. Relation between electrolyte imbalance and excretion of an anti-diuretic substance in adrenalectomized cats. *Am. J. Physiol.* 126: 51. 1939.

The purpose of the investigation was to determine, if an antidiuretic principle is excreted during adrenal insufficiency, what is its relationship to electrolyte imbalance. Twelve cats were used in the study, 180 rats being used for the bioassays for diuretic principle in urine by the method of Burn. Fluid intake and urine output were measured in the cats under 5 experimental regimens i.e., (1) control period, (2) 24 to 72 hr. of dehydration (3) after adrenalectomy on maintenance dose of adrenal extract, (4) 24 to 72 hr. after cessation of extract injections, (5) on restoring the animals' serum sodium to normal with adrenal extract (in a few animals). No significant variations in serum Na or K were found accompanying the excretion of the antidiuretic imbalance during the first 24 to 72 hrs. of dehydration in cats. Adrenalectomized cats maintained on adequate doses of adrenal extract did not excrete an antidiuretic substance. However such substance was found in the urine 24 hr. after cessation of extract therapy. Its appearance at this time was largely due to decreased intake, and not to changes in serum Na or K. Forty-eight to 72 hr. after extract was discontinued and after a definite electrolyte imbalance was noted, much larger quantities of the antidiuretic substance were excreted. Decreased serum Na in conditions other than hypoadrenia (paracentesis) similarly results in excretion of large amounts of the antidiuretic substance. It is believed that the functions of the adrenal cortex and the posterior pituitary are related directly insofar as they are both concerned with water metabolism. Following adrenalectomy, there is an initial stimulation of the posterior pituitary due to early dehydration and a secondary augmentation caused by electrolyte imbalance.—K. A. Brownell.

MASSELIN, J. N. Influence of light and darkness on the melanophore dilating action of the hypophysis (Influencia de la luz y la oscuridad sobre la acción melanófora de la hipófisis). *Rev. Soc. argent. de Biol.* 15: 28. 1939.

The hypophysis of toads was extracted in saline and injected subcutaneously into toads; variations in skin color were observed when compared with a control. The glands obtained in winter had stronger melanophore dilating activity than those obtained in summer. Keeping the animals in the dark the activity of the gland increased after 11 to 14 days and went on increasing to a maximum with longer time of darkness. Strong, permanent illumination and a white background decreased the activity of the gland after 11 to 14 days. Extirpation of the eye-balls produced the same effect as darkness; a strong light playing on the head of these blind animals diminished the activity of the hypophysis. Electrical stimulation of the optic nerves had the same effect as strong light on the eye. There was no difference in the activity of hypophyseal extracts made with saline, distilled water and NaOH 0.1 N. The blood from 6 toads, in various conditions of illumination, was injected intravenously into a normal toad in the course of 1 hr. drawing 5 cc. of blood and injecting the same amount every 10 min. This procedure had no effect on the melanophores.—J. T. L.

MASSELIN, J. N. Histology of the hypophysis of toads kept in light and darkness (Histología de la hipófisis de sapos sometidos a oscuridad y luz). *Rev. Soc. argent. de Biol.* 15: 35. 1939.

In winter the *Bufo arenarum* anterior pituitary lobe had 70.4% cromophobe cells, 26.2% acidophile and 3.4% cyanophile. In spring and summer there were cromophobes 34.15%, acidophile 50.7% and cyanophile 15.15%. After 8 days in darkness in winter the acidophile cells increased to 54.2%, with a corresponding decrease in the cromophobe cells. This variation was not observed after 4 days of darkness and its persisted after 30 and 60 days of darkness. In summer toads the acidophile increase due to darkness was established earlier, the maximum was observed at 4 days and persisted for at least 16 days. Strong light and a white background increased the cromophobe cells with a corresponding decrease in the acidophile. In summer the reaction occurred earlier and was more marked than in winter. Extirpation of the eye-balls acted like darkness. Electrical stimulation of the optic nerves produced the effects of light. —J. T. L.

MATTHEWS, S. A. The relationship between the pituitary gland and the gonads in Fundulus. *Biol. Bull.* 76. 241. 1939.

Alkaline extracts of whole sheep pituitary, injected into the body cavity of males, had no effect on the gonads. Extracts of whole pituitary of sheep, extracts of fish pituitary and antuitrin-S were injected into 35 females. In 4 cases, eggs were delivered earlier than in the controls. Of the hypophysectomized animals, weight records and fixed gonads were obtained from 33 males and 40 females. When the pituitary was removed in the fall, the testes did not enlarge as they normally do in the following May or June. Removal of the pituitary in March or April, also produced a smaller testis. The pituitary exerts more influence on the maturation than on the proliferation of the germ cells.—E. L.

MELCHIONNA, R. H., AND R. A. MOORE. The pharyngeal pituitary gland. *Am. J. Path.* 14: 763. 1938.

Material from 54 autopsies was studied. Only those cases in which the autopsy was performed a few hours after death were chosen; otherwise there was no selection of cases. A small mass of typical

or atypical pituitary tissue was found in the pharyngeal mucosa in 51 out of the 54 cases. The differentiated cells in the pharyngeal pituitary were found to be histologically identical with those in the anterior lobe of the pituitary gland but there were relatively few chromophilic cells. It is concluded that under normal conditions of growth and activity it is unlikely that the pharyngeal pituitary gland contributes any significant physiological function, but that in some cases of altered structure or function of the pituitary it may undergo alterations and serve as an endocrine gland.—W. J. A.

Melville, K. I. Cardiac response to posterior pituitary extract as affected by sodium phenobarbital. *J. Pharmacol. & Exper. Therap.* 66: 107. 1939.

Postlobin-V, 1.5 U per kg. in dogs anesthetized with phenobarbital, caused ventricular fibrillation which could be prevented by the previous administration of adrenaline, ephedrine or papaverine. From electrocardiographic and isolated heart studies, it is evident that phenobarbital disturbs ventricular conductance in addition to predisposing the coronaries to constriction by post-pituitary extract.—E. L.

Meyer, R. K., C. H. Mellish and H. S. Kupferman. The gonadotropic and adrenotropic hormone o the chicken hypophysis. *J. Pharmacol. & Exper. Therap.* 65: 104. 1939.

Pituitaries were removed from the heads of male and female chickens and suspended by grinding in distilled water. Graded amounts were injected into normal and hypophysectomized immature female rats. The male hypophyses contained more gonadotropic hormone than the female glands. Luteinizing hormone was present in the glands from both sexes. The gonadotropic effect was enhanced by heme. An adrenotropic hormone was also present in this suspension but no lactogenic hormone could be demonstrated.—E. L.

Meyler, L. Simmonds' disease (Hypophysary emaciation). *Acta Med. Scandinav.* 96: 157. 1938.

A review of the literature on Simmonds' disease was given and the case histories of 13 women who had the condition were reported. All cases showed a lowering of the B.M.R. ranging from 15 to 45%, amenorrhoea and emaciation. Most of the cases also had a low blood-sugar level and a low blood pressure, with a dryness of the skin and a loss of hair. Treatment with thyroid, pituitary hormone or pregnancy urine by enema gave good results in a few of the cases but in others was without effect. Transplantation of the pituitary gland though not used by the author appeared to be the most promising method of treatment.—J. M. L.

Renander, A. Cutis verticis gyrata one of the symptoms of acromegaly. *Acta Med. Scandinav.* 96: 186. 1938.

A description was given of the pathological conception cutis verticis gyrata and its resemblance to familial acromegaly pointed out. Following x-ray treatment to the hypophysis, one case showed considerable regression of the hyperplastic alterations in the connective tissue of the skin. The conclusion was made that the condition is not a separate disease entity but a symptom of acromegaly.—J. M. L.

PANCREAS

Adlersberg, D., and H. Dolger. Medico-legal problems of hypoglycemia reactions in diabetes. *Ann. Int. Med.* 12: 1804. 1939.

Diabetic individuals, taking insulin, may present in hypoglycemia a wide variety of behavioristic and mental changes foreign to their normal personality. The legal problems involved are discussed.—From Authors' Summary.

Allen, F. M. Tolerance and toxicity of insulin. III. Protamine and zinc compounds. *Ann. Int. Med.* 12: 1870. 1939.

The tolerance of animals for protamine zinc insulin is lower than for regular insulin. The duration of the hypoglycemic effect of the former is up to 5 days in rats and 8 days in rabbits. This prolonged effect is most closely imitated by small doses of regular insulin repeated at very short intervals. Amputation experiments prove that protamine insulin is retained at the site of the injection as long as 62 hours. Protamine insulin does not have the same 'toxic' action as regular insulin.—F. A. H.

Bowcock, H. A discourse upon problems in the differential diagnosis and treatment of diabetes mellitus with a discussion of the protamine paradox. *Internat. Clin.* 1: 110. 1939.

This paper is of such scope as to be incapable of satisfactory abstracting. The author suggests that a more thoughtful and experimental attitude could be adopted by the clinician toward the following problems: differential diagnosis, pregnancy, individualization in diet, the greatest success in therapy.—I. B.

CLAY, H. L., AND H. LAWSON. The relationship of the hyperglycemic principle in diabetic urine to the pancreas and the hypophysis. *Am. J. Physiol.* 125: 566. 1939.

Using rabbits as test animals the presence of a blood sugar raising principle in human diabetic urine has been confirmed. An extract having a similar effect was obtained from the urine of 19 out of 22 pancreatectomized dogs. No such substance could be found in the urine of normal dogs. Maintenance of pancreatectomized dogs on insulin and raw pancreas did not cause the disappearance of the principle, but it failed to appear in the urine of 4 out of 5 dogs that were pancreatectomized after hypophysectomy and it disappeared from the urine of 1 pancreatectomized dog after hypophysectomy.—F. A. Hitchcock.

CORNIL, L., J. E. PAILLAS AND H. ROSANOFF. Hypoplasia of the islets of Langerhans in dogs injected with testosterone propionate (Sur l'hypoplasie des ilots de Langerhans des chiens injectés au propionate de testostérone). *Compt. rend. Soc. de biol.* 129: 981. 1938.

Four dogs, 2 male and 2 female, received daily subcutaneous dose injections of 1 mg. of testosterone propionate for 2 weeks. When they were killed 2 weeks later, there was an almost complete disappearance of the islet tissue.—J. C. D.

DOHAN, F. C., AND F. D. W. LUKENS. Persistent diabetes following the injection of anterior pituitary extract. *Am. J. Physiol.* 125: 188. 1939.

Permanent diabetes was produced in 3 of 8 dogs by the administration of progressively larger doses of AP extract (est. of 10 to 35 gm. of tissue over a period of 30 days). The results were similar to those of Young and of Campbell and Best. While the intensity of the diabetes varied the metabolic behavior of the animals was like that of partially depancreatized dogs. Histological evidence, also, indicated damage to the islands of Langerhans.—K. A. Brownell.

DUNCAN, G. G. The treatment of diabetes mellitus during the course of acute infections and surgical complications. *Internat. Clin.* 2: 31. 1939.

Diabetes per se is not a fatal disease. It is the acute medical or surgical condition complicating this chronic and incurable disease that kills. The 3 obvious methods of reducing the number of deaths due to diabetes plus complications are: the prevention of diabetes; the prevention of complications in diabetics and improvement in the treatment of acute complications. One hundred and thirty-six diabetics were treated for acute complications at the Pennsylvania and Jefferson Hospitals, Philadelphia. In 13 of the 23 fatalities diabetes was considered a contributary cause of death. The author concludes that acute infections and surgical complications are major causes of death in this disease. A simple method of treating diabetes during the course of acute complications with equal division and distribution of the diet and insulin is described.—I. B.

JOACHIM, H., AND M. M. BANOWITCH. A case of carcinoma of the islands of Langerhans with hypoglycemia. *Ann. Int. Med.* 11: 1754. 1938.

A case of spontaneous hypoglycemia is reported in which the entire syndrome ran its course in 2 months. At operation a tumor of the pancreas which proved to be a carcinoma of the islets of Langerhans was found.—From the Authors' Summary.

JOSLIN, E. P., AND P. WHITE. Diabetes in children. *J. Ped.* 12: 255. 1938.

There are about 12,000 diabetic children under the age of 20 years and 8000 under the age of 15 years in this country at present, whereas before the insulin 'era' there were 1000 cases per year. There are 50 reported cases in the literature of acquired diabetes under one year. In preinsulin days 1000 diabetic children per year died in the United States; today 1 child in 100 dies per year. According to a recent study of the Metropolitan Life Insurance Company, a child afflicted with diabetes at the age of 10 years and treated has a life expectancy of 31.7 years. The causes of death of diabetic children are diabetic coma, pulmonary tuberculosis, infections, hypoglycemia and accidents. The treatment of the new born infant of a diabetic mother is described. If diabetics marry and have children, 24% of the offspring will develop diabetes; this will occur in 10% of crosses between diabetics and carriers and in 4% of crosses between carriers. Several complications met with in diabetic children are coma, loss of resistance to infections, premature development and degenerative changes, deficiency disease, metabolic disturbance of the skin, dwarfism and hepatomegaly. The treatment with protamine insulin is discussed. The round table conference of which this paper was a part should be read in the original; it is difficult to abstract in full.—M. B. G.

LICHENSTEIN, A. Free diet in children with diabetes. *J. Ped.* 12: 182. 1938.

The author advocates an absolutely free diet without any restrictions and without weighing the food or any of its ingredients. Insulin is given in doses sufficient to keep the child in good general condition, in good increase of weight and free from ketone bodies in the urine. Little importance is attached to the amount of sugar excretion, unless there is an associated change for the worse in the general condition.

The author has used this treatment in about 50 cases in children ranging in age between 2½ to 16 years for periods of ½ to 4 or 5 years with favorable results.—M. B. G.

LUNDSGAARD, E., N. A. NIELSEN AND S. L. ORSKOV. On the possibility of demonstrating an effect of insulin on isolated mammalian liver. *Skandinav. Arch. f. Physiol.* 81: 11. 1939.

The addition of insulin failed to affect the utilization of glucose by the perfused, isolated rabbit's liver. The experiments were performed under conditions of varying oxygen saturation of the arterial blood, during the continuous administration of glucose, and during the inhibition of glucose utilization by the continuous administration of epinephrine.—A. G.

McCULLAGH, E. P. Protamine-zinc-insulin in diabetes. *Ann. Int. Med.* 11: 1979. 1938.

Protamine-zinc-insulin in common dosage appears to be active for 50 to 65 hours after injection. Its maximum effect is usually maintained between 12 and 18 hours. The prolonged effect of single daily doses makes it unnecessary usually to use more than one dose daily. The cumulative action increases to the 3rd day or longer and reduces the requirement of insulin to less than 75% of the dose of regular insulin in most cases. A normal fasting blood sugar level marks the limit of increase in protamine-zinc-insulin dosage. If, when this limit is reached, the blood sugar levels during the day remain high, one or more accessory doses of regular insulin are required for control.—From the Author's Summary.

McINTYRE, A. R., AND J. C. BURKE. Vitamin B fractions and insulin tolerance in the albino rat. *J. Pharmacol. & Exper. Therap.* 65: 36. 1939.

Insulin, 20 U per kg. daily for 23 days, had no effect on the rate of weight gain of rats (120 to 220 gm. in weight) fed on an adequate diet. After 21 days on a diet deficient in vitamin B, the same dose of insulin caused deaths. The heat-stable portion of the vitamin B complex protected the rats from ill effects of insulin but vitamin B_1 (thiamin) increased the sensitivity to insulin.—E. L.

MACKAY, E. M., R. H. BARNES AND H. C. BERGMAN. Influence of insulin on protein metabolism as measured by the nitrogen balance. *Am. J. Physiol.* 126: 155. 1939.

Nitrogen retention of albino rats was increased by the administration of 4 or 10 U of insulin per day. The increased retention occurred whether the food intake was mixed or the diet supplied ad libitum.—F. A. Hitchcock.

MALAMUD, N., AND L. C. GROSH, JR. Hyperinsulinism and cerebral changes. *Arch. Int. Med.* 61: 579. 1938.

Malamud and Grosh report the case of a woman aged 30 who had had hypoglycemic reactions for 6 years. Fainting spells, which were relieved by ingestion of food or sweets, gradually merged into convulsive attacks and a concomitant change in the mental status, characterized by irritability, resistiveness, impulsiveness and, finally, gradual but progressive deterioration. At necropsy an encapsulated adenoma of the islet cells was seen. The brain weighed 1,125 gm. The leptomeninges were thin and moderately congested. The cerebral cortex showed generalized parenchymatous degeneration, most pronounced in the left hemisphere and affecting chiefly layers 3 and 5. In some areas the degeneration extended also into layers 2 and 6, whereas the fourth layer was usually well preserved. In the first layer there was considerable gliosis. The white matter was essentially normal, with only slight rarefaction in the myelin sheaths and moderate increase in the number of glial fibers.—Abstract from *Arch. Neurol. & Psychiat.*

MARSHALL, C., W. S. McCULLOCH AND L. F. NIMS. pH of the cerebral cortex and arterial blood under insulin. *Am. J. Physiol.* 125: 680. 1939.

About 1 hour after curarized animals are given intravenous injections of insulin under constant artificial respiration fluctuations in the pH of the cortex appear without corresponding fluctuation in the pH of the arterial blood. Intravenous injections of buffered dextrose stops these fluctuations and lowers the pH of both the cortex and arterial blood.—F. A. Hitchcock.

MIRSKY, I. A. The influence of insulin on the protein metabolism of nephrectomized dogs. *Am. J. Physiol.* 124: 569. 1938.

Using the rate of the accumulation of N.P.N. in the blood of bilaterally nephrectomized dogs as an index of protein catabolism observations were made on nephrectomized animals with and without insulin, completely eviscerated animals with and without insulin and eviscerated animals given intravenously 600 mg. of lycine per kg., with and without insulin. There were from 7 to 10 animals in each group. Amino acid nitrogen and N.P.N. of the blood was determined in all experiments. The administration of insulin seemed to result in a decrease in the rate of protein catabolism of the nephrectomized dogs, a decrease in the rate of amino acid excretion from the muscles of eviscerated dogs and an increase in the utilization of exogenous amino acids by the muscles of eviscerated dogs. It is suggested that insulin exerts

its protein sparing action by decreasing deamination in the liver and by increasing amino acid utilization by the muscles.—F. A. Hitchcock.

PHILLIPS, R. A., AND S. B. BARKER. Insulin convulsions after the removal of the stellate ganglia. *Am. J. Physiol.* 124: 202. 1938.

Insulin convulsions were observed in 3 cats and in 2 dogs both before and after the removal of both stellate ganglia. These results contradict those of Drabkin and Ravdin (1937).—F. A. Hitchcock.

RATHERY, F., AND J. TURIAF. Influence of castration and injections of testosterone on the islet tissue in the pancreas of the guinea pig (Influence de la castration et des injections de testostérone sur les ilots de Langerhans du pancreas du cobaye). *Compt. rend. Soc. de Biol.* 128: 155. 1938.

Five guinea pigs were killed from 2 to 11 weeks after castration. The islet tissue showed an increase in both the size and number of islands. In 5 uncastrated animals, injected with testosterone for 10 days, no change in the islet tissue could be detected.—J. C. D.

RUSSELL, J. A. The action of insulin and of anterior pituitary extract in normal and hypophysectomized rats. *Am. J. Physiol.* 124: 774. 1938.

An investigation was made of the effects of insulin and of AP extract given with insulin, on the metabolism of carbohydrate in normal and hypophysectomized rats, in both fasted and fed states. Approximately 400 animals were used. Very small doses of insulin (0.05 to 0.10 U/kg.) profoundedly reduced the O_2 consumption of fasted, hypophysectomized rats, whereas larger doses (up to 3 U/kg.) produced only small changes in the metabolic rate of normal rats. An alkaline extract of anterior pituitary in appropriate dosage prevented the hypoglycemic action of insulin in both normal and hypophysectomized rats. The APE was also able to prevent completely the depression of O_2 consumption produced by insulin in hypophysectomized rats. It was considered tentatively that the contra-insulin effects of APE were best explained as produced partly by preventing the withdrawal of glucose from the blood as muscle glycogen and partly by its action in depressing carbohydrate oxidation.—K. A. Brownell.

ROUSSY, G., AND M. MOSINGER. The neuro-endocrine structure of the pancreas and its stimulation by extracts of the anterior lobe of the pituitary (Sur la neurocrinie pancréatique et sa stimulation par l'extrait antéhypophysaire). *Compt. rend. Soc. de Biol.* 126: 1064. 1937.

Five adult guinea pigs received daily injections of anterior lobe extract for a period of 6 weeks. The islet tissue was hyperplastic. The new tissue grew from modified aciner tissue and was most extensive in the interlobular septa. Here, the endocrine cells were in close contact with the nerve trunks and ganglion cells. The islet tissue seemed to be actually secreting. This intimate association of endocrine and nervous tissue resembles the condition found in the posterior part of the hypophysis and suggests the possibility that vagotonin may have a neuro-endocrine origin.—J. C. D.

SAHYUN, M., A. NIXON AND M. GOODELL. Influence of certain metals on the stability of insulin. *J. Pharmacol. & Exper. Therap.* 65: 143. 1939.

Separate portions of insulin were incubated for 7 weeks at 52° C. with zinc 0.05, 0.1 or 0.4 mg., or cobalt, nickel and aluminum 1 mg. per 100 U. Samples assayed weekly showed that the physiologic activity was preserved by these metals.—E. L.

TOLSTOI, E., AND F. C. WEBER, JR. Protamine zinc insulin: A metabolic study. *Arch. Int. Med.* 64: 91. 1939.

Two young women with severe diabetes were carefully observed for 50 and 60 days respectively. Their caloric intake was constant at 1,640 calories, and each received a single dose of protamine zinc insulin in the morning. In one case the dose was 50 and in the other 60 U. The composition of the diet was 75 gm. of protein, 60 gm. of fat and 200 gm. of carbohydrate for the first 30 days, and this diet was divided into equal portions at times (⅓, ⅓, ⅓) and into unequal portions at other periods. After this 30-day period the diet was changed to 75 gm. of protein, 105 gm. of fat and 100 gm. of carbohydrate. This reduced the carbohydrate by ½, but the total calories were kept constant by increasing the fat. Equal and unequal fractionation of this diet also was employed. The dose of insulin was always kept the same, and the effects of these procedures were observed. It was noted that the patients excreted an average of 100 gm. of dextrose daily during the first experimental 30-day period, in spite of which they were remarkably free from any and all symptoms of diabetes and their loss of weight was no greater than could be accounted for by their diet, which was subcaloric for them as judged by the Aub-Du Bois standards. The authors conclude to the effect that when protamine zinc insulin is used to treat diabetes the glycosuria is not materially influenced by dietary shifts from equal to unequal portions. With protamine zinc insulin, the glycosuria was diminished by a lower carbohydrate diet, but the utilization of dextrose was greater with the higher carbohydrate diets. For patients treated with protamine zinc insulin the guiding feature of satisfactory treatment should be the maintenance of weight, freedom from symptoms

and absence of ketone bodies in the urine. Glycosuria is desirable, as it affords protection from reactions. —I. B.

VARTIAINEN, I., AND L. BASTMAN. The retardation of the effect of insulin by means of arginine. *Acta. Med. Scandinav.* 98: 318. 1939.

Although arginine alone in amounts up to 100 mg. per kg. did not affect the blood-sugar levels of rabbits it did cause a retardation of the action of insulin from 10 to 13 hours, similar to the effect of protamine. The addition of zinc chloride to the arginine insulin mixture caused a still greater delay in the insulin action, 12 to 16 hours.—J. M. L.

VOGELENZANG, E., AND L. A. HULST. Die Bedeutung des Zinkgehaltes von Handelinsulin (The significance of the zinc content in commercial insulin). *Acta Med. Scandinav.* 97: 307. 1938.

A change in the dosage of insulin needed to control the diabetes in 5 patients was found to be related to the amount of zinc in the preparations. The zinc content of various samples of insulin supplied at different times varied from 0.005 mg. to 2.04 mg. per 1000 units. The lowest value was obtained in January, 1938 and the highest in April. The dosage of insulin required had to be diminished as the zinc content increased. The author recommends a stabilizing of the zinc content of all insulin preparations.—J. M. L.

WEITZE, M. The action of insulin in suckling mice. *Acta Med. Scandinav. Supplement* 90: 111. 1938.

Young suckling mice 1 to 15 days old were injected with 0.025 I.U. of insulin and kept at 36° C. for 10 hours or more. The survival period is nearly the same for mice 1 and 2 days old, but the mortality is higher for the younger animals. The blood sugar determined in mice 1 to 2 days old fasting from 1 to 4 hours showed marked variations from 100 mg. to 30 mg. per 100 cc. of blood. The response in suckling mice differed from that in adults in that it appears later and is almost always fatal while 55% of adult mice recover. The late effect is not due to diet, carbohydrate stores, nor low temperature and it cannot be prevented by glucose injection.—J. M. L.

YANNET, H., with the technical assistance of J. F. IANNUCCI. Experimental study of pathogenesis of cerebral changes following prolonged insulin hypoglycemia. *Arch. Neurol. & Psychiat.* 42: 395. 1939.

The present study is an investigation in cats of some factors considered responsible for the production of cerebral injury following prolonged insulin hypoglycemia. The mechanism for the cerebral damage was not found to be causally related to changes in oxygen saturation of arterial blood or to the presence of marked circulatory impairment. It was found that relatively small doses of insulin, i.e., 3 U per kg., resulted in prolonged hypoglycemia but did not produce cerebral injury. However, larger doses, i.e., from 15 to 20 U per kg., did result in widespread cerebral damage, although the hypoglycemia produced was neither more prolonged nor more severe as judged by the concentrations of the blood sugar. These results were interpreted as suggesting that the injurious effects of large doses of insulin are brought about by the promotion of some reaction in the cerebral cells which is relatively harmless if the blood sugar is maintained. Animals under anesthesia induced by pentobarbital sodium were found to be more susceptible to the toxic action of insulin.—Author's Summary.

PARATHYROID

BAUMANN, E. J., AND D. B. SPRINSON. Hyperparathyroidism produced by diet. *Am. J. Physiol.* 125: 741. 1939.

Hyperparathyroidism in rabbits has been produced by feeding a low Ca high P (carrot and oats) diet. This diet causes enlargement of the parathyroids to two or more times their normal size and hypertrophy of the cells and nuclei with an increase in the amount of lipids in the cytoplasm. Serum Ca and P are low but within the normal range, while serum phosphatase is possibly less than normal. Aside from increased vascularity no important changes were found regularly in the bones. The Hamilton and Schwartz test for parathormone is always high.—From the Authors' Summary.

CANTAROW, A., AND V. G. HAURY. Diffusion of calcium, magnesium and phosphorus into the peritoneum. The effect of intravenously injected calcium salts and of parathyroid hormone. *Am. J. Physiol.* 126: 66. 1939.

In order to overcome objections to the ultra-filtration and cerebrospinal determinations as a measure of diffusible serum Ca, the authors studied the equilibrium between serum and 50 to 100 cc. of 2.5% dextrose in 0.9% NaCl injected into the peritoneal cavity of normal, unanesthetized dogs. It was thought that such fluid might be representative of interstitial fluids in general. The test period lasted 24 hours. Equilibrium with serum Ca and P was reached in 4 to 5 hours after introduction of fluid and remained so

for the balance of the 24-hour period. Intravenous injection of Ca gluconogalactogluconate resulted in a progressive increase in fluid Ca, lagging behind the change in diffusible serum Ca (determined by ultrafiltration) but finally equaling the diffusible Ca 1 to 2 hours after injection of the salt. During the period of decreasing serum Ca the fluid Ca decreases more slowly than ultrafiltrate Ca. After injection of 500 to 600 U of parathyroid hormone into normal dogs the fluid Ca and the serum Ca both increased, the ratio of fluid Ca to serum Ca remaining constant for about 5 hours. Then as the serum Ca decreases the ratio rises sharply. The changes in fluid Ca lag behind changes in ultrafiltrate Ca by approximately 1 hour. Under certain conditions (washing out of Ca by repeated paracentesis) parathyroid hormone may result in a fall of serum Ca. However fluid Ca always increases. Since this occurs in the face of a falling serum Ca the fundamental effect of hormone on serum Ca appears to be to increase its diffusion from the blood into the interstitial fluid.—S. R. Tipton.

CHUTE, R. Clinical aspects of hyperparathyroidism with special reference to urology. *J. Urology.* 41: 726. 1939.

Based on 36 proven cases of hyperparathyroidism at the Massachusetts General Hospital, the clinical aspects of this disease are discussed with special reference to urology. Hyperparathyroidism is rare, less than 1 case per 1500 general hospital admissions, and is the etiological factor in about 3% of cases of urinary calculi. Women are affected more than twice as often as men. The average age is 43. In addition to general symptoms of weakness, there are symptoms secondary to lithiasis in the urinary tract, and symptoms due to decalcification of the bony skeleton. Eighty-three per cent of cases had urinary lithiasis. In 53% calculi were bilateral, and in 73% multiple. Calculi are composed chiefly of calcium phosphate. Diagnosis is established by demonstration of elevated serum calcium (over 11½ mg. per 100 cc). Sulkowitch test for amount of calcium excretion in urine is quick, easy and valuable. Surgical resection is the only treatment that has been successful. End results in this series have been satisfactory. No new calculi have occurred in any case. The urologist must think of the possibility of hyperparathyroidism in every case of urinary lithiasis.—Author's Summary.

CLARK, J. H. The effect of parathyroid hormone on the permeability of the lens capsule to calcium. *Am. J. Physiol.* 126: 136. 1939.

A KCl extract of lens protein from pig eyes is denatured and becomes opalescent when exposed to ultraviolet radiation for 35 minutes at 4 C. and then subsequently heated to 40 C. for 40 minutes, if the lens contains Ca. The degree of opalescence varies with the amount of Ca in the lens. The lenses are immersed in Ringer's for 24 hours at icebox temperature. Controls show considerable opalescence when tested. Ultra-violet radiation, PH, viosterol and ascorbic acid had no effect on the lens permeability to Ca. Parathyroid hormone (Lilly) added to the Ringer's completely depressed the penetration of Ca into the lens. Fresh lens gave the same effect. If Ca of Ringer's is increased more hormone is necessary to prevent penetration. The author suggests that parathyroid hormone prevents the penetration of Ca into body cells; cataracts occurring after parathyroidectomy may be due to a combination of denaturation of the lens protein if eye is exposed to ultra-violet radiation, and greater permeability of lens to Ca in the absence of parathyroid hormone.—S. R. Tipton.

DRAKE, R. G., F. ALBRIGHT, W. BAUER AND B. CASTLEMAN. Chronic idiopathic hypoparathyroidism; report of six cases with autopsy findings in one. *Ann. Int. Med.* 12: 1751. 1939.

The criteria necessary for a diagnosis of chronic idiopathic hypoparathyroidism are low serum Ca and high serum inorganic P levels, normal bone texture by roentgenogram, and the absence of renal insufficiency. Eight cases of the disease collected from the literature are cited and 6 new cases are added. The autopsy findings are given in one fatal case. The parathyroid glands were normal in size but histologically all the epithelial cells were entirely replaced by fat cells, a finding heretofore not recorded in parathyroid pathology. The possibility is mentioned that idiopathic hypoparathyroidism may be the antithesis as regards the parathyroid disorder of idiopathic hypertrophy of the parathyroids with hyperparathyroidism. Attention is called to the fact that the disease usually starts in childhood or adolescence and, if not then, not until about forty. In several instances the onset was closely related to acute infections. The possibility of some bacterial or virus infection injuring the glands is discussed.—From Authors' Summary.

EISLER, A. B. Functional hyperplasia of the parathyroids in experimental anaemias. *Brit. J. Exper. Path.* 19: 342. 1938.

Experiments were done to show the effect of toxic anemia upon the parathyroid glands of rabbits. The anemia was produced by the injection of hydroxylamine over a long period of time. Results showed that the average weight of the parathyroid gland in the anemic rabbits was increased over that of normal ones by about 15.8 mg. This increase, unaccompanied by renal damage, was thought to be secondary to certain changes in the calcium metabolism.—Margaret Baptist.

GUTMAN, A. B., AND W. B. PARSONS. Hyperparathyroidism simulating or associated with Paget's disease; with three illustrative cases. *Ann. Int. Med.* 12: 13. 1938.

Three proved cases of hyperparathyroidism are described, 2 presenting sclerotic lesions simulating Paget's disease, of the pelvis in one instance and of the skull in the other; and the 3rd case apparently associated with Paget's disease of the tibia and skull.—From the Authors' Summary.

PATRAS, M. C., E. A. GALAPEAUX AND R. D. TEMPLETON. Dietary management of albino rats before and after thyroparathyroidectomy. *Am. J. Physiol.* 122: 409. 1938.

The effect of the salt content of diets and of the injection of ammonium salts on the development of tetany and the death rate following thyroparathyroidectomy in rats was determined. In all, 796 rats 23 to 38 days old were used; the environmental temperature of the animals was kept at 88–92° F. $Ca(OH)_2$ was found to be superior to $CaCl_2$ as an inorganic dietary salt for the prevention of tetany during the first 48 hours following thyroparathyroidectomy. The injection of NH_4C_4 decreased the mortality and morbidity in the operated animals. The combination of calcium salt and ammonium salt treatments produced the most beneficial effects. When the salt intake of the animals was reduced during a 10-day preoperative period, the incidence of tetany and death following thyroparathyroidectomy was increased. This latter effect was much less pronounced for older rats (203 animals 100 days old, and 23 animals 150 days old), presumably because in the older animals the body is better supplied with protective stores. The authors therefore believe that the variability in mortality and morbidity of thyroparathyroidectomized rats is due rather to variations in the pre- and post-operative diets than to possible accessory parathyroid tissue.—S. L. Cohen.

RICHTER, C. P., AND J. F. ECKERT. Mineral appetite of parathyroidectomized rats. *Am. J. M. Sc.* 198: 1939.

Parathyroidectomized rats showed a markedly increased appetite for calcium solutions (lactate, acetate, gluconate and nitrate) and an aversion toward dibasic sodium phosphate solution. Parathyroidectomized rats also showed an increased appetite for strontium and magnesium salt solutions. These results agree with the present knowledge concerning the disturbed calcium and phosphorus metabolism after parathyroidectomy. The decreased mortality and alleviation of deficiency symptoms of parathyroidectomized rats given access to calcium solutions add further proof that rats have ba ability to make selections conducive to their well-being.—Authors' Summary.

ROJAS, P., AND F. J. MANFREDI. Histophysiology of parathyroid grafts (Histofisiología del injerto glandular paratiroideo). *Bol. Acad. Nac. Med. Buenos Aires* 303. 1938.

Experiments in dogs. Grafts implanted in muscles did not take, they were reabsorbed by the histiocytic reaction of perimuscular connective tissue. Under the renal capsule on the kidney parenchyma was found a most favorable location. The rich capillary bed supplied rapid vascularization and the fibrous nature of the capsule made the histiocytic reaction of small importance. Autoplastic grafts 'took' in 90% and homoplastic in 30% of the cases. Grafts were observed microscopically in section from 4 hours up to 16 months after grafting. Their survival was dependant on vascularization, this occurred in 24 hours and was completed in 4 days; in 20 days the tissue's aspect became definitely stabilized. A few hours after operation the outer cells of the graft had a normal aspect, while towards the center the cells showed signs of degeneration. Later (40 to 48 hours) the center became necrosed; on the 4th day capillaries invaded this amorphous zone; on the 7th day a lax connective tissue had replaced it, which became stable scar tissue by the 20th day. The internal layer of cells of the outer zone started to multiply, at about the 90th day there were numerous mitotic figures and cordons of cells were formed which penetrated into the connective scar tissue. In the course of the 6th or 7th month the process was completed and the gland reconstructed. Grafts which suffered reabsorption sometimes disappeared rapidly in 10 hours, no glandular cells were recognisable, an inflammatory process followed. In grafts included in muscles a slower form was sometimes observed, granulation tissue gradually absorbed the glandular tissue. Grafts 'took' more readily in parathyroidectomized animals. Blood Ca and P determinations showed the functional capacity of these grafts.—J. T. Lewis.

THYMUS

CHIODI, H. Effect of castration before puberty on the thymus (Action de la castration prépuberale sur le thymus). *Compt. rend. Soc. de biol.* 129: 866. 1938.

Ninety-two male white rats were castrated at 30 days. They and their litter-mate controls were kept under carefully standardized conditions. Rats were killed at ages varying from 45 days to 250. The normal curve for absolute thymus weight rises sharply to about 80 days and then starts to decline. In the castrated rats, the weight of the thymus was above that of the controls but the curve had the same general shape as that for normals. The involution of the thymus, therefore, is not dependent on sexual maturity or the presence of the testis.—J. C. D.

LEBLOND, C. P., AND G. SEGAL. Action of poison on the thymus of adrenalectomized animals (Action des toxiques sur le thymus de l'animal sans surrénale). *Compt. rend. Soc. de biol.* 129: 838. 1938.

Nine controls and 28 experimental rats were used. Some were simply poisoned with x-ray or colchicine, while others were similarly treated at varying times after adrenalectomy. The latter operation tends to produce an enlargement of the thumus. Lack of adrenals does not, however, prevent the poisons from producing their usual involutional effect on the thymus, though not to the extent found in unoperated animals.—J. C. D.

WASSERMAN, P., AND J. W. EPSTEIN. Congenital carcinoma of the thymus with extensive generalized metastases: *J. Ped.* 14: 798. 1939.

A case of congenital primary carcinoma of the thymus with metastases to skin, lungs and liver, is reported. The mother had congenital syphilis and the child presented skin lesions resembling lues but serological and slide tests were negative. Post-mortem findings are described in detail.—M. B. G.

THYROID

BEAUMONT, G. E., AND J. D. ROBERTSON. Anginal syndrome due to hypothyroidism. *Lancet* 1: 682. 1939.

The authors describe a case which was originally diagnosed as angina of effort. No clinical evidence was found, but the B.M.R. was −33. Under adequate treatment with thyroid extract the patient was enabled to return to work and his anginal symptoms disappeared. With either an overdosage or an underdosage of thyroid extract his symptoms recurred. The case emphasizes the fact that subtotal thyroidectomy for the relief of angina may incur the danger of increasing the symptoms unless myxedema and 'masked hypothyroidism' have been excluded.—From *J. A. M. A.*

BROWN, A. W., I. P. BRONSTEIN AND R. KRAINES. Hypothyroidism and cretinism in childhood; VI Influence of thyroid therapy on mental growth. *Am. J. Dis. Child.* 57: 517. 1939.

The authors report on a further study of the results of treatment with thyroid over a period of from 1 to 7 years of 29 children with cretinism and 4 with juvenile hypothyroidism. On the basis of mental testing by several methods, they conclude that most cretins with an I.Q. below 70, with a mental age at maturity of 10 to 11 years, remain severely retarded, but a small proportion may develop normally. There appears to be some relation between development of carpal bones and mental age. Early recognition of the condition and persistent treatment are important factors in the ultimate mental development.—M. B. G.

CASTLEMAN, B., AND S. HERTZ. Pituitary fibrosis with myxedema. *Arch. Path.* 27: 69. 1939.

The authors report a case of destructive fibrosis of the anterior lobe of the pituitary gland without cachexia. On the contrary, the patient not only was in a good state of nutrition but had myxedema. At necropsy, fibrosis of the anterior lobe of the pituitary and atrophy of the thyroid, parathyroids, adrenal glands, ovary and uterus were observed. The authors present pathologic and experimental evidence purporting to establish the primacy of the anterior lobe of the pituitary in the pathogenesis of the disease. From the microscopic observations alone the diagnosis in this case would be Simmonds's disease, but the absence of cachexia raises the question whether the cases in which there is no cachexia represent a special disease group. Pluriglandular insufficiency would probably be a more accurate clinical designation. The lesion in the pituitary gland was that of destruction of most of the cells of the anterior lobe plus lymphocytic infiltration. The lesion in the thyroid was devoid of inflammatory elements.—From *Arch. Neurol. & Psychiat.*

DENNIG, H. The non-operative treatment of hyperthyroidism. *Ann. Int. Med.* 11: 1472. 1938.

A review of the non-operative treatment of hyperthyroidism is presented—physical, psychic, dietetic treatment and especially the effect of iodine and roentgen-radiation. Their limits and indications are discussed.—Author's Summary.

GAEBLER, O. H., AND P. BARTLETT. Metabolic effects of growth and thyrotropic hormones administered with and without iodine. *J. Ped.* 13: 936. 1938.

In experiments on dogs, daily records were kept of weight, water intake, urine volume, excretion of total nitrogen, creatinine and creatine and the basal metabolism. Addition of 1 cc. of Lugol's solution to the daily ration did not significantly alter the nitrogen storage or calorigenic effect resulting from single injections of growth preparation. Marked storage of creatine accompanied the storage of nitrogen. Single injections of thyrotropic hormone produced just detectable calorigenic effects, but produced a marked creatinuria which was not prevented by administration of iodine, and differed in this respect from the creatinuria of human hyperthyroidism. The animals become refractive to the action of the thyrotropic hormone on creatinuria after as few as 3 small injections at 10-day intervals.—M. B. G.

GIEDOSZ, B. Influence of vitamin B_1 on the thyroid (Sur l' influence de la vitamine B_1 sur la glande thyroïde). *Compt. rend. Soc. de biol.* 129: 333. 1938.

Evidence obtained in studies on guinea pigs led to the conclusion that B_1 is not antagonistic to thyroxine, that it stimulates the thyroid gland, and that it does not interfere with the action of thyrotropic hormone.—E. Cutuly.

GOLDBERG, S. J. Circulation time as a diagnostic aid in hyperthyroidism. *Ann. Int. Med.* 11: 1818. 1938.

The calcium gluconate method of determining circulation time was employed in 72 patients with hyperthyroidism. The average rate was found to be 8.8 ± 0.9 seconds as contrasted with an average reading of 12.5 ± 1.0 seconds in 60 normal subjects. Evidence is presented to indicate that the circulation time test is useful in the diagnosis of hyperthyroidism.—From the Author's Summary.

KLAPPER, H. Exophthalmic goiter in a child five years of age. *Internat. Clin.* 2: 234. 1939.

The case reported occurred in a male child 5 years old with symptoms analogous to those of exophthalmic goiter in adults. Thyroidectomy wsa performed in 3 stages with apparent relief of symptoms. —I. B.

KRICHESKY, B. The influence of thyroidectomy on the period of gestation in the rabbit. *Am. J. Physiol.* 126: 234. 1939.

Thyroidectomy performed 1½ hours to 12 days after mating (52 does) caused a decreased rate of O_2 consumption (14 to 38% below that of 15 control animals), but had no effect on the length of the period of gestation or in the histology of the ovaries of the rabbits. Fourteen of the animals mated a 2nd time with a subsequent second normal gestation period. Seven of the 52 experimental animals did not pass through a normal delivery; these animals either died at the time of delivery or exhibited paralysis of the hind limbs immediately after delivery. Since no attempt had been made to preserve the parathyroid glands during thyroidectomy, it is possible that parathyroid hormone may be important in the normal parturition processes.—S. L. Cohen.

MAHAUX, J. Reversal of thyrotropic hormone action on basal metabolism in rabbits after combined injections of thyrotropic hormone and thyroxine (Inversion de l'action de l' "hormone thyréotrope" sur le métabolisme basal du lapin après injection simultanée d' "hormone thyrétrope" et de thyroxine). *Compt. rend. Soc. de biol.* 129: 39. 1938.

The effect of thyrotropic hormone upon B.M.R. was determined in rabbits previously injected with thyroxine or with thyroxine and thyrotropic hormone. Thyroxine injected before thyrotropic hormone actually produced an effect opposite to that resulting from thyroid stimulation, but did not abolish exophthalmos. The same result was obtained when thyroxine and thyrotropic hormone preceded an injection of thyrotropic hormone alone. An interpretation of the results, pointing to blockage of central secretory centers, is included.—E. Cutuly.

MARONEY, J. W., AND J. A. JOHNSTON. Effect of thyroid on nitrogen and calcium in the growth period. *J. Ped.* 13: 937. 1938.

Continuous Ca and N balances with concurrent B.M.R. determinations were made on a cretin to whom thyroid was administered and an adolescent with hyperthyroidism whose gland was removed. Retention of both Ca and N was increased with the administration of thyroid. The child with hyperthyroidism showed a negative Ca balance which became positive with thyroidectomy. However when the B.M.R. fell to −20% following the removal of both lobes, a slightly positive Ca balance became definitely more positive with the administration of thyroid. The improvement was referable to a diminished stool loss. The negative balance in the hyperthyroid state was associated with a high urinary output. A similar anabolic effect was noted in the case of nitrogen.—M. B. G.

MITCHELL, C. L., AND J. A. JOHNSTON. A continuous calcium and nitrogen balance in a case of Legg-Perthes' disease treated with growth hormone and thyroid. *J. Ped.* 13: 937. 1938.

A continuous Ca and N balance for 66 days with concurrent basal metabolism determinations was obtained on a boy with Legg-Perthes' disease. The Ca balance was negative on an average intake, was not appreciably changed by growth hormone, but was made definitely positive by thyroid. The B.M.R. was low. The intercurrence of an acute throat infection rendered the balance again negative, but following its subsidence, it became positive again with thyroid. Clinically the patient improved rapidly. —M. B. G.

RAWLS, W. B., A. A. RESSA, B. GRUSKIN AND A. S. GORDON. Thyroid activity in chronic arthritis. *Ann. Int. Med.* 11: 1401. 1938.

Thyroid extract produced improvement in a small proportion of chronic arthritics.—F. A. H.

Ring, G. C. Thyroid stimulation by cold (including the effects of changes in body temperatures upon basal metabolism). *Am. J. Physiol.* 125: 244. 1939.

Physiological evidence is given to show that long continued exposure to cold increases the release of thyroid secretion. The basal metabolism of 5 normal rats was elevated by an average of 21% after living for 3 weeks or more in a refrigerator at 0° to 5° C. If the thyroid glands were completely removed, the animals did not survive in the cold. Three partially thyroidectomized rats showed little elevation of metabolism (6%) as a result of cold. Corrections made for elevation in body temperature of normal rats after living in the refrigerator still leave a large increase in metabolism (16%) to be accounted for on the basis of thyroid stimulation. The pathways for this stimulation do not require the superior cervical ganglia since their removal does not modify the results.—Author's Abstract.

Rose, E., Atypical manifestations of hypothyroidism. *Pennsylvania M. J.* 42: 752. 1939.

In addition to clinical manifestations which are frequently atypical, the diagnosis of true hypothyroidism depends upon (a) reduction in basal metabolism, (b) increase in blood cholesterol, (c) response to therapy, and (d) in children, roentgen evidence of delayed bony development. The optimal dose of desiccated thyroid varies widely, and can be determined for the individual only by cautious trial. The various commercial preparations of thyroid gland on the market vary in potency. Hence, it is important to adhere to one brand throughout the patient's course of treatment.—I. B.

Sharpless, G. R. Relation of diet to the experimental production of goiter. *J. Ped.* 13: 937. 1938.

In 5 to 7 weeks the thyroid gland is enlarged to 4 times normal in rats fed on a diet of raw soy bean flour, yeast, butterfat, sugar and salts. Iodine will prevent the enlargement, but the minimum requirement is greater than that for rats fed on other diets. Treating the flour by prolonged heating or extracting with solvents reduces its goiterogenic activity even though the iodine content of the diet may be reduced. —M. B. G.

Sherwood, T. C. The relation of estrogenic substance to thyroid function and respiratory metabolism. *Am. J. Physiol.* 124: 114. 1938.

Thyroidectomy resulted in a reduction of 30% in the basal heat production (33 Cal./sq.m./hr.) of 19 albino rats. The oral administration of 1 gm. of desiccated thyroid substance per kg. per day for 3 days produced an increase of 79% in 8 controls and 75% in 11 experimental animals. The experimental animals were injected with 1000 I.U. of amniotin per day for 3 days immediately following the thyroid administration. The heat production of the experimental animals returned to the hypothyroid level in about 6 days while in the control group this return required about 24 days. It is concluded that there is a direct hormone antagonism or a direct tissue effect.—F. A. Hitchcock.

Simon, A. Myxedematous hypothyroidism and psychosis. *Ill. Med. J.* 75: 66. 1939.

Three cases of myxedema with psychosis are reported. Young persons who develop myxedema and who possess a schizoid personality are prone to develop schizophrenic psychoses. Elderly patients with involutional and cerebral arteriosclerotic changes may develop an organic psychosis characterized by a loosely constructed paranoid delusional system, with confusion and vague hallucinatory experiences. Appropriate thyroid therapy may improve the physical and possibly ameliorate the mental condition.—I. B.

Smelser, G. K. A comparative study of experimental and clinical exophthalmos. *Am. J. Ophth.* 20: 1189. 1937.

Biopsies of the orbital tissues in 6 cases of exophthalmos were studied; 5 had undergone thyroidectomy and 1 had an oxygen consumption rate of 131% of prediction. The retrobulbar connective tissue fat, and muscles were edematous and infiltrated with wandering cells. The extraocular muscles were enlarged, though muscle-fiber degeneration was not striking. In a series of experimental studies in about 100 thyroidectomized guinea pigs to which AP extract was administered exophthalmos due to increased orbital tissue was produced. The retrobulbar connective tissue, fat, and muscles were infiltrated with edematous material indistinguishable from that in the clinical cases. The exophthalmos persisted after death. Removal of the cervical sympathetic ganglion did not inhibit the increase in orbital tissues. Areas of round-cell infiltration were found in the fat and muscles.—R. G. H.

Smelzer, G. K. Treatment of experimentally produced exophthalmos with thyroxin and other iodine compounds. *Am. J. Ophth.* 21: 1209. 1939.

Injection of guinea pigs (in adequately large numbers) with alkaline extract of beef anterior pituitary glands gave rise to exophthalmos. Coincident administration of NaI or diiodotyrosine did not influence the exophthalmos. Thyroxin caused exophthalmos and reduced the incidence of pituitary-exophthalmos from 86 to 52%. Exophthalmos was more readily produced in hyperthyroid than in hypothyroid animals. The data were shown statistically to justify the conclusions cited.—R. G. H.

SOSKIN, S., R. LEVINE AND R. E. HELLER. Rôle of the thyroid in the carbohydrate disturbance which follows hypophysectomy. *Am. J. Physiol.* 125: 220. 1939.

Five totally hypophysectomized dogs were treated with 0.5 mg. thyroxin per kg. per day, their fasting blood sugars determined by the Somogyi modification of the Shaffer-Hartman method and urinary nitrogens by the macro-Kjeldahl procedure during fast periods of as long as 3 weeks. The effect of insulin on these animals was also determined by injecting 1/40 to 1/10 U per kg. The intramuscular injection of thyroxin into totally hypophysectomized dogs maintained a normal blood sugar level throughout long periods of fasting, increased their urinary nitrogen to that of fasting normal animals but did not diminish their hypersensitivity to insulin. The secondary atrophy of the thyroid probably plays an important part in the decreased protein catabolism and in the related carbohydrate disturbance of the hypophysectomized animal. Thyroid atrophy is apparently not the cause of insulin sensitivity.—K. A. Brownell.

THOMPSON, W. O., The hypothyroid states. *Internat. Clin.* 23: 175. 1939.

Hypothyroidism may be primary or secondary. The primary form is caused largely by destruction of thyroid tissue, the secondary by lack of adequate stimulation of the thyroid by the anteior pituitary and possibly also by the adrenal cortex. Treatment depends upon the apparent cause of the syndrome. It is necessary in all cases to bear in mind that starvation and nephrosis may lower the basal metabolic rate. In some patients a singularly low basal metabolic rate is apparently normal to the individual. Hypothyroidism is commonly associated with Addison's disease and may be so marked that myxedema may develop.—I. B.

TILLGREN, J., AND N. SUNDGREN. Hyperthyroidism and Lugol treatment. *Acta Med. Scandinav.* 98: 1. 1938.

A summary in detail of 44 cases of hyperthyroidism treated with Lugol's solution is given. Good results were obtained in 17 cases, partial effects in 7 cases, and transitory action in 2 cases. In 5 cases repeated effects after discontinuing and resuming treatment two to three times were observed. Five of the patients died during treatment, one of these from typical Basedow's cachexia and 2 from heart failure.—J. M. L.

TILLGREN, J., AND N. SUNDGREN. Hyperthyroidism and Lugol treatment. *Acta Med. Scandinav.* 98:172. 1939.

Detailed case reports are given of 25 cases of hyperthyroidism in which operation was performed. In 16 cases pre-operative treatment with Lugol's solution was given with good results, and in 5 cases Lugol's solution and x-ray treatment were used. Two of the patients died, one from bronchopneumonia and the other from a toxic crisis. Pronounced post-operative reactions occurred in 4 cases and hyperthyroid symptoms were found in 3 cases after operation.—J. M. L.

TILLGREN, J., AND N. SUNDGREN. Hyperthyroidism and Lugol treatment. *Acta Med. Scandinav.* 98: 289. 1939.

A comparison of the results of 200 cases of hyperthyroidism of which 120 were treated medically and 80 surgically. The chief symptoms noted were palpitation, nervousness, goiter and loss of weight. In the medically treated series there were 7.5% of deaths directly attributable to the thyroid condition as against 6.3% in those operated upon. The frequency of operation increases in proportion to the rise in B.M.R.—J. M. L.

TURNER, K. B., AND A. STEINER. A long term study of the variation of serum cholesterol in man. *J. Clin. Investigation* 18: 45. 1939.

Ten patients with conditions which often lead to hypercholesterolemia were studied over a year, with many determinations of cholesterol. There is no significant change in serum cholesterol during the day, or after feeding, even when the feeding contains much cholesterol. The administration of 2 gm. KI daily does not change the level. Thyroid, given to the point of a moderate elevation in basal metabolism caused a distinct reduction in cholesterol, which had returned to the usual level by 6 weeks after withdrawal. The adminstration of high fats diets caused no significant rise in cholesterol, nor did the inclusion in the fat intake of 10 gm. cholesterol daily for 6 weeks. Save for the effect of thyroid the cholesterol level seems characteristic of the individual plus his chronic disorder, with variations of about 10% from the mean.—E. L. S.

WAHLBERG, J. The Roentgen treatment of thyrotoxic edema occurring during sleep (Röntgenbehandlung bei thyreotoxischem Schlafenödem). *Acta Med. Scandinav. Supplement* 89: 209. 1938.

A case is described of a 21-year-old girl who showed massive edema of the temporal regions during sleep. The author described a correlation between this effect and the hypophyseomesencephalic relationship of the thyroid gland. Exposure of the hypophyseal region to ½ the erythema dose of x-rays caused improvement of the condition.—J. M. L.

WAHLBERG, J. Thyroid diseases in Finland (Studien Über die Schilddrüsenkrankheiten in Finland). *Acta. Med. Scandinav. Supplement* 94: 1938.

This is a 295-page monograph on thyroid disease as it is seen in Finland. It is based on a study of 2,350 cases. The first section deals with the endemiological, etiological, and prophylactic aspects of the condition. The next section describes the histology and histophysiology of the thyroid gland in health and disease. The next three sections give in detail the diagnosis and treatment of simple enlargement and adenoma, the hypothyroid conditions and the thyrotoxicoses. The final two sections deal with thyroiditis, both acute and chronic, and malignant tumors.—J. M. L.

WEGELIN, C. The hypophysis in exophthalmic goiter. *Ann. d'anat. path.* 15: 703. 1938.

After the discovery of the hypophysial thyrotropic hormone, the theory was advanced that exophthalmic goiter may be due to hypersecretion of this hormone. Wegelin studied the hypophysis in 20 cases of this disease, but in none of them did he find evidence of hyperfunction of the anterior lobe. The outstanding change was always more or less pronounced pericapillary edema with marked hyperemia. Degenerative changes, in the form of diffuse swelling or complete degeneration, were observed in the three types of cells of the anterior lobe. The injection into a guinea pig of acetone extract of the hypophysis from a patient with exophthalamic goiter did not change the epithelial lining of the thyroid gland. The injection of the extract of a normal human hypophysis was followed by the production of cuboid epithelium lining the thyroid follicles and by diminution in the amount of colloid.—From *Arch. Neurol. & Psychiat.*

WEGELIN, C. Pathogenesis of intralaryngotracheal goiter. *Schweiz. med. Wchnschr.* 69: 593. 1939.

Wegelin was able to connect the theories of von Bruns and Paltauf, for he observed that intralaryngotracheal goiter occurs in fetuses and newborn infants and that even at this stage there exists a connection between the external and internal portion of the thyroid. He found that the bridge of tissue is between the cricoid cartilage and the first tracheal cartilage and that it infiltrates the cricotracheal membrane. Moreover, he was able to observe a preliminary stage of the intralaryngotracheal goiter; in a newborn child and in a child aged 5 years he detected thyroid tissue in the submucosa of the trachea and in the cricotracheal membrane, although these sites showed no macroscopic swelling. In these cases the external thyroid was greatly enlarged. On the basis of these observations the author assumes that in case of goitrous hyperplasia the thyroid may grow into the larynx and trachea during the fetal period but that a later invasion is likewise possible. In studies on goiter in white rats the author was able to make observations which corroborate Paltauf's opinion. The animals received only cooked foods (oats and maize gruel) and some bread. They developed either diffuse or nodular goiters and the author observed that an intralaryngotracheal goiter develops when the enlarged thyroid penetrates between the cricoid and tracheal cartilages and spreads under the mucosa. All stages of invasion could be demonstrated. Since such changes are absent in normal thyroids, their presence seems to indicate that in human subjects also intralaryngotracheal goiter is caused chiefly by the goitrous proliferation of the thyroid, during either fetal or extra-uterine life.—*From J. A. M. A.*

WERNER, A. A., J. LEWALD, G. A. JOHNS AND D. KELLING. Growth in children with mongolism; a four-year study of eight patients. *Am. J. Dis. Child.* 57: 554. 1939.

The authors report the results of a study of 8 children with mongolism treated with an AP extract containing the growth factor and with desiccated thyroid. The stature was approximately 5 inches under that of normal children of the same age. The children grew in height and weight approximately as did untreated normal children of the same age over a 4-year period. Five untreated children with mongolism increased in height and weight at a rate approximately equal to that of normal children of the same age and over the same period of time. The mental age and intelligence quotient were not improved by treatment.—M. B. G.